Anemia and erythropoietin in cardiovascular disease

Kleijn, Lennaert

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Lennaert Kleijn

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1 Introduction and aim of the thesis

Chapter

Cardiovascular disease (CVD) is to date the number one cause of death worldwide, accounting for almost 30% of all global deaths. Despite development of novel therapeutics it is projected that death from CVD will increase to 23.3 million in 2030.[1] Major disorders in the group of CVD include coronary heart disease, cerebrovascular disease, congenital heart disease and thrombotic and embolic disease. Eventually, cardiovascular disease can cause a state of heart failure (HF), a complex of symptoms related to impaired cardiac function. The Framingham study showed that the current lifetime risk to develop heart failure is 1 on 5.[2]

Anemia and cardiovascular disease

Anemia is an important co-morbidity which is frequently observed in patients with cardiovascular disease.[3-6] Most commonly it is defined by decrease of hemoglobin as a reflection of the pathological state of reduced circulating red blood cells.[7] Its presence in patients with cardiovascular disease has been associated with significantly impaired morbidity and mortality and therefore understanding its cause is of clinical relevance. Causes of anemia generally divide in decreased production, increased destruction or loss of red blood cells. In cardiovascular disease, anemia is caused mainly by decreased production of red blood cells. Under normal conditions, a hormone to maintain hemoglobin at a constant level, erythropoietin (EPO) is secreted in response to low oxygen levels in the kidney. This in turn causes erythrocyte maturation and growth. Several factors are associated with decreased red blood cell production. The renin-angiotensin-aldosterone-system (RAAS) is frequently activated in patients with cardiovascular disease due to hypoperfusion. This subsequently causes renal vasoconstriction in order to maintain its filtration rate. Fluid retention occurs, as a result of vasopressin and antidiurectic hormone, leading to hemodilution.[8] Other less well understood causes in cardiovascular disease are iron deficiency and bone marrow impairment, although the latter can be caused by bone marrow resistance to erythropoietin. This is further fuelled by the observation of disproportionally high erythropoietin levels in heart failure patients. These may be explained by either anemia, RAAS activation and increased levels of inflammatory factors. [9,10]

Erythropoietin in cardiovascular disease

Discovered first as hematopoietine in patients with high red blood cell counts living on height, its name was replaced by erythropoietin in 1948.[11] After purification and cloning of the hormone, its use was first registered for patients with renal anemia.[12] As more patients were using erythropoietin, patients treated with the hormone showed increase

in cardiac function. With the discovery of the erythropoietin receptor in different organs than the red bone marrow, it was hypothesized that erythropoietin could possess non-erythropoietic effects as well.[13] Indeed, in experimental settings, erythropoietin decreased myocardial infarct size in experiments of ischemia and reperfusion. Second, erythropoietin improved cardiac function in models of exeperimental heart failure independent of infarct size trough increasing capillary to myocyte ratio.[14] Eventually, the mechanism of erythropoietin was found due to mediated by upregulation of vascular endothelial growth factor (VEGF) and increased endothelial progenitor cells, leading to this increased capillary density and increased cardiac performance.[15]
Furthermore, erythropoietin was responsible for decrease in apoptosis of cardiomyocytes exposed to ischemia.[16] To date, several large clinical studies have been performed to assess erythropoietin treatment to either preserve cardiac function or correct anemia in an attempt to increase cardiac function and reduce mortality and morbidity.[17] The results of these trials will be discussed in this thesis and put into perspective.

Aims of this thesis

The first part of the current thesis is focused on the etiology of anemia in patients with cardiovascular disease, including coronary artery disease, heart failure and patients undergoing coronary artery bypass graft (CABG) surgery. In **chapter 2** we studied the correlation between hemodynamic parameters and hemoglobin levels in a broad spectrum of cardiovascular patients. **Chapter 3** focuses on inflammation in patients with chronic heart failure. Heart failure is characterized by increase levels of cytokines, which may influence erythropoiesis and EPO production. In this chapter we establish the association between anemia and inflammation in heart failure patients. In **chapter 4** we studied the prognostic significance of sustained post-operative anemia in patients following CABG surgery and the role of RAAS inhibition in this process. In **chapter 5** we tried to further elucidate the relation between inflammation and anemia. Therefore we studied the association between the bone marrow response (reticulocyte count) to anemia and inflammation in patients before and after CABG surgery.
The second part comprises the role of erythropoietin treatment in CVD. In **chapter 6** we discuss the potential role of erythropoietin in heart failure patients based on a recently published meta-analysis. During short term follow up there was a trend towards lower event rate in patients with acute MI treated with erythropoietin. In **chapter 7** we present the long term effects of erythropoietin on cardiovascular endpoints. In **chapter 8** we give comments on the current state of erythropoietin therapy in heart failure patients. **Chapter 9** summarizes this thesis and provides future perspectives on the origin and treatment of anemia in patients with CVD.

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Part 1 | Anemia in cardiovascular disease

Chapter 2

Anemia and its association with hemodynamics in a broad spectrum of cardiovascular patients

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Abstract

Background:

Anemia is frequently observed in patients with cardiovascular disease. Multiple factors have been associated with anemia, but the role of hemodynamics is largely unknown. Therefore, we investigated the association between hemoglobin (Hb) levels, hemodynamics and outcome in a broad spectrum of cardiovascular patients.

Methods:

A total of 2009 patients who underwent right heart catheterization at the University Medical Center Groningen, the Netherlands, between 1989 and 2006 were identified and data were extracted from electronic databases. Anemia was defined by the WHO criteria (male: hemoglobin < 13.0 g/dL, female: hemoglobin < 12.0 g/dL). The associations between central venous pressure (CVP), cardiac index (CI), systemic vascular resistance (SVR), hemoglobin (Hb), anemia and all cause mortality were assessed with linear, logistic and Cox-proportional hazards analysis.

Results:

The mean age was 57 ± 15 years, 57% were male, mean Hb was 8.3±0.3 g/dL, and 27.4% of the patients were anemic. Patients with anemia had higher CVP levels (7.0±5.4mmHg) compared to non-anemic patients (5.6±4.1 mmHg; p<0.001). CI was slightly higher in anemic patients; 3.0±2.9 L/min/m^2 vs. 2.9±0.8 L/min/m^2 (p<0.001), whereas SVR was significantly lower (1212±479 dyn x sec x cm^{-5} vs. 1356±555 dyn x sec x cm^{-5}, p<0.001). CVP and CI were both independent predictors of anemia (OR 1.49; CI1.24-1.81, p<0.001 and OR: 1.93; CI1.54-2.42, p<0.001, respectively). Hemoglobin and CVP were both independent predictors of survival. Patients with anemia and an elevated CVP had the worst prognosis (HR 2.17; 95%CI 1.62-2.90; p<0.001). The impact of elevated CVP and anemia on prognosis was independent of CI and renal function.

Conclusion

Anemia is common in cardiovascular patients and independently related to an elevated CVP and CI. Patients with both elevated CVP and anemia have the worst prognosis, independent of cardiac index.

Background

Anemia is a comorbidity frequently observed in cardiovascular patients.[1] Its presence is independently associated with morbidity and mortality in a broad range of cardiovascular diseases, including heart failure, myocardial infarction and patients with suspected angina. [1-6]

The etiology of anemia in cardiovascular patients is mostly studied in heart failure (HF) cohorts. These observations have shown that causes for anemia are multifactorial. Several mechanisms may contribute to lower hemoglobin (Hb) levels[7], such as an inadequate production of erythropoietin (EPO)[8], chronic kidney failure[9], haematinic abnormalities[10], use of medication[11], and bone marrow dysfunction[12,13]. Elevated levels of cytokines also play a role in the anemia observed in patients with HF.[14] Although not all of these factors have been studied, similarities exist in the etiology of anemia in patients with other cardiovascular diseases. For instance, in acute coronary syndromes, inflammatory factors may play an import contributing role.[15]

There is only limited data available on the association between hemodynamics and hemoglobin levels. Most data come from experimental studies performed in the 1970s and 1980s. It has been shown that acute isovolumic anemia results in an increased cardiac output (CO) and heart rate (HR), and a reduced systemic vascular resistance (SVR).[16-18] The few studies appearing on chronic severe anemia show that the increased CO is mostly accomplished by increasing stroke volume.[19,20] However all these studies have been performed in experimental settings and data in human subjects, especially the association with outcome, are lacking. In the current study we investigated the association between hemodynamic parameters, hemoglobin levels and outcome in a broad spectrum of cardiovascular patients with varying etiologies.

Methods

Case identification

Using the patient registration system of the University Medical Center Groningen, The Netherlands, all patients that underwent right heart catheterization between January 1, 1989, and December 31, 2006 were identified. All performed intracardiac measurements were extracted from the patients file. Only unique cases were used. The study was performed following the UMCG research code and approved by the local medical ethical committee.

Data extraction

Retrospective chart review was performed to analyze characteristics of all patients that were identified during the electronic search as previously described.[21] For each patient, date of birth, sex, race, weight and height were collected. Comorbid conditions, including hypertension, coronary artery disease, cardiac valve disease, congenital heart disease, history of stroke, hypercholesterolaemia, and diabetes, in addition to medical treatment at the time of catheterization were extracted. Survival status was determined using the electronic patient registration database of the University Medical Center Groningen. Follow up started at the moment of catheterization. The primary endpoint of interest was all cause mortality.

Heart catheterization

Hemodynamic variables obtained during catheterization included systolic blood pressure (SBP; mm Hg), diastolic blood pressure (DBP; mm Hg), CO (thermodilution, L/min), PCWP (mm Hg) and right atrial pressure as indicator of CVP (CVP, mm Hg). Cardiac index (CI; L/min/m^2) was determined as cardiac output divided by the body surface area, which was calculated as: $0.007184 \cdot weight^{0.425} \cdot length^{0.725}$. SVR (dyn x sec x cm^{-5}) was calculated as mean arterial pressure minus CVP times 80, divided by cardiac CO. Measurements obtained from cardiac catheterization were obtained from the patient during a resting state.

Laboratory measurements

Routine laboratory assessments at catheterization were extracted from the electronic registration database. If no lab was available at the day of catheterization, most recent measurements were taken within three months prior to catheterization.

Definitions

Anemia was defined according to the WHO criteria as Hb<13 g/dL for men and Hb<12 g/dL for women. To define elevated CVP, we dichotomized CVP at the highest quartile representing a cut-off value of 8 mm Hg. Furthermore, cardiac dysfunction was defined as a cardiac index below 2.5 L/min/m^2, as previously described.[22]

Statistical analysis.

Results are presented as mean ± standard deviation (SD) when normally distributed and as median and interquartile range (IQR) when skewed distributed and as numbers and percentages for categorical variables. Differences between groups were compared

with Student's t-test, χ^2-test or Mann-Whitney U testing where appropriate. The relation between CVP and Hb or anemia was assessed with standard linear or logistic regression analysis respectively. The variables age, gender, CVP, CI, eGFR, heart rate, SVR, DBP, SBP, history of DM, history of heart transplant, congenital heart disease as reason for catheterisation, diuretics use, Angiotensin Converting Enzyme (ACE) inhibitors or Angiotensin Receptor Blocker (ARB) use, Mineralocorticoid Receptor Antagonist (MRA) use were assessed for their univariate association with Hb or anemia. Variables that showed a significant ($p<0.10$) univariate association were manually entered in a stepwise multivariable model based on the strength of their univariate.association. A cox regression analysis was performed with interaction analsyis of Hb and CVP. Variables that showed a significant association, were manually entered in a stepwise backward multivariable model based on strength of univarate associaction. Multivariable associated variables were adjusted for all univariable associated variables. In addition interaction between Hb and CVP was assessed in this model.

Kaplan-Meier survival plots were constructed to display the influence of anemia and increased CVP on all cause mortality. The association between anemia, CVP, cardiac dysfunction and all cause mortality was assessed by Cox proportional hazards regression analysis. Univariate hazard ratio (HR) and 95% confidence interval (95%CI) of death from any cause were calculated for CVP, anemia or both. Multivariable Cox regression models were then constructed to study the effect of CVP, SVR, CI and anemia on

Table 1. Indications for right heart catheterization

Indication	Percentage of patients (%)
Heart Failure	18.8
Aortic Valve Stenosis	16.3
Coronary Artery Disease	13.8
Mitral Valve Insufficiency	13.4
Pre Transplantation	12.5
Rhythm Disorder	5.3
Aortic Valve Insufficiency	5.3
Pulmonary Hypertension	3.3
Post Heart Transplantation	2.4
Mitral Valve Stenose	1.0
Pulmonary Valve Insufficiency	0.8
Pulmonary Valve Stenosis	0.3
Other	6.8

Table 2. Baseline characteristics.

Variable	All patients (n=2009)	No Anemia (n=1458)	Anemia (n=551)	P value
Age (yrs)	57 ± 15	57 ± 15	59 ± 16	0.014
Female gender (%)	855 (43)	627 (43)	228 (41)	0.245
SBP (mm Hg)	131 ± 29	130 ± 28	127 ± 32	0.005
DBP (mm Hg)	68 ± 13	69 ± 12	65 ± 15	< 0.001
CO (L/min)	5.4 ± 1.6	5.4 ± 1.5	5.6 ± 1.7	0.028
CI (L/min/m2)	2.9 ± 0.8	2.9 ± 0.8	3.0 ± 2.9	< 0.001
CVP (mm Hg)	6.0 ± 4.5	5.6 ± 4.1	7.0 ± 5.4	< 0.001
Heart rate (beats/min)	79 ± 17	78 ± 17	81 ± 18	0.001
Stroke volume (ml)	72 ± 24	72 ± 24	72 ± 25	0.6107
SVR (dyn x sec x cm-5)	1320 ± 550	1356 ± 555	1212 ± 479	< 0.001
PVR (dyn x sec x cm-5)	128 ± 110	126 ± 110	132 ± 108	0.714
eGFR (ml/ min/ 1.73m2)	65 ± 25	68 ± 22	56 ± 31	< 0.001
Medical History (%)				
Heart failure	332 (18)	240 (18)	92 (20)	0.637
Coronary artery disease	414 (23)	296 (22)	118 (26)	0.130
Congenital heart disease	99 (5)	83 (6)	16 (3)	0.030
Valvular disease	828 (46)	616 (46)	212 (46)	0.953
Hypercholesterolemia	258 (14)	183 (14)	75 (16)	0.815
Diabetes Mellitus	153 (9)	97 (7)	56 (12)	0.003
Hypertension	363 (20)	263 (20)	100 (22)	0.528
Stroke	92 (5)	63 (5)	29 (6)	0.168
Medication (%)				
Diuretics	743 (41)	508 (38)	235 (51)	< 0.001
Beta-Blocker	500 (28)	374 (28)	126 (27)	0.720
ACEi or ARB	696 (39)	496 (37)	200 (43)	0.032
MRA	159 (9)	101 (8)	58 (13)	0.001

Values are mean ± SD or n (%) unless listed otherwise.. SBP = systolic blood pressure; DBP = diastolic blood pressure; CO = cardiac output; CVP= central venous pressure; eGFR = estimated glomerular filtration rate; SVR = systemic vascular resistance; PVR = pulmonary vascular resistance; ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker. MRA = Mineralocorticoid receptor antagonist. Values are corrected for age and gender

mortality after adjusting for predictors of mortality in HF (age, gender, eGFR, diuretics use, ACE/ARB use, MRA use, coronary artery disease, diabetes mellitus and reasons for catheterization aortic valve insufficiency, pre-transplantation, heart failure or rhythm disturbances). The assumption of proportional hazards was assessed by graphing the HR according to their category after multivariable interaction analysis. All tests were 2-tailed and a p-value<0.05 was considered statistically significant. All analyses were performed with STATA version 12.0.

Results

Demographics

Between 1989 and 2006, a total of 3757 right heart catheterizations were performed.

Table 3. Univariate and multivariable predictors for hemoglobin levels.

Variable	Univariate		Multivariable	
	beta	P-value	beta	P-value
Age	-0.129	< 0.001	- 0.066	0.028
Gender	0.246	< 0.001	0.202	< 0.001
eGFR	0.257	< 0.001	0.213	< 0.001
CVP	-0.158	< 0.001	-0.181	< 0.001
Cardiac Index	-0.137	< 0.001	-0.263	< 0.001
DBP	0.175	< 0.001	0.154	< 0.001
History of Diabetes	-0.107	< 0.001		
Diuretic Use (Yes)	-0.138	< 0.001		
ACEi or ARB use	-0.049	0.039		

SBP = systolic blood pressure; DBP = diastolic blood pressure; CO = cardiac output; CVP= central venous pressure; eGFR = estimated glomerular filtration rate; DM = Diabetes Mellitus ACE = angiotensin-converting enzyme; ARB = angiotensin receptor blocker. Values are corrected for age and gender.

Of these, 2557 (68%) were first or only right heart catheterizations of unique patients. Patients having no hemoglobin levels available (n=549) were excluded. Our final study population contained 2009 subjects. Indications for right heart catheterization are shown in table 1.

Baseline demographics of the patient population according to the presence or absence of anemia are presented in table 2. Mean age was 57 ± 15 years and 43% were female. Anemia was present in 27.4%, an elevated CVP in 20.6% and 31.8% of the patients had evidence of cardiac dysfunction, defined as cardiac index of less than 2.5 L/min/m^2. Anemic patients had significantly lower systolic and diastolic blood pressures, a

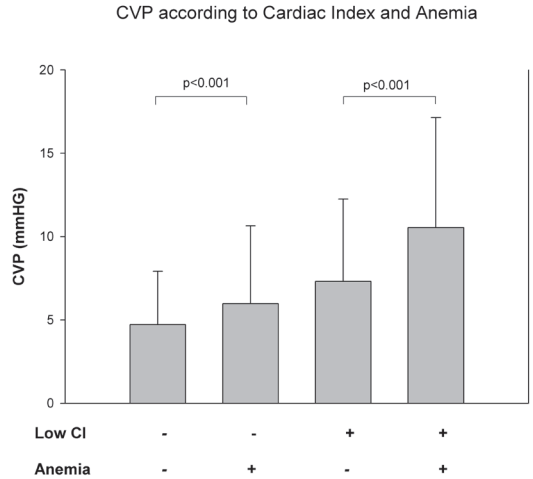

Figure 1. Schematic representation of Central venous pressure in different groups. CVP= Central Venous Pressure; CI= Cardiac index.

lower GFR and more frequently had a history of diabetes. Other comorbidities were comparable between anemic and non-anemic patients. Patients with anemia were more often using diuretics, ACE inhibitors and MRAs compared to non-anemic patients. Heart rate was significantly higher in anemic patients. Anemic patients had a significant higher CVP and CI, whereas SVR was lower. Stroke volume was comparable between anemic and non-anemic patients. Increased CVP was observed in anemic patients and this was most pronounced in patients with a low cardiac index (figure 1).

Association between hemoglobin, anemia and CVP.

Table 3 shows the linear regression analysis on univariable and multivariable predictors of hemoglobin levels. Adjusted for age and gender, hemoglobin levels were significantly correlated with eGFR, CVP, CI, diastolic blood pressure, history of DM, diuretic use and ACEi or ARB use. There was no significant correlation between SVR and hemoglobin levels. In multivariable analysis, CVP and CI remained independent predictors of hemoglobin levels.

Table 4 shows the outcomes of a multivariable logistic regression analysis for determinants of anemia. The multivariable regression model showed that CI, eGFR,

Table 4. Predictors of anemia in logistic regression

Variable	Univariate OR (95% CI)	P-value	Multivariable OR (95% CI)	P-value
Age	1.01 (1.00 – 1.01)	0.02		
Gender	0.99 (0.81 – 1.20)	0.91		
CVP per 5 mmHg	1.36 (1.23 – 1.52)	< 0.001	1.49 (1.24 – 1.81)	< 0.001
CI per l/min/m^2	1.28 (1.12 – 1.46)	< 0.001	1.93 (1.54 – 2.42)	< 0.001
eGFR (per 10 mL/min/1.73m^2)	0.83 (0.79 – 0.86)	< 0.001	0.85 (0.79 – 0.92)	< 0.001
Heart rate (per 10 bpm)	1.13 (1.06 – 1.20)	0.001	1.12 (1.01 – 1.24)	0.03
SBP (per 10 mmHg)	0.96 (0.92 – 1.00)	0.04		
DBP (per 10 mmHg)	0.79 (0.72 – 0.86)	< 0.001	0.79 (0.66 – 0.94)	0.009
Medical History				
Diabetes Mellitus	1.79 (1.27 – 2.53)	0.001		
Reason for Catheterisation				
Congenital heart disease	0.25 (0.09 – 0.71)	0.015		
Post HTx	3.94 (2.13 – 7.29)	< 0.001	7.44 (2.08 – 26.6)	0.002
Medication				
ACEi or ARB	1.28 (1.04 – 1.59)	0.02		
Diuretics	1.64 (1.33 – 2.03)	<0.001		
MRA	1.73 (1.23 – 2.43)	0.002		

SBP = systolic blood pressure; DBP = diastolic blood pressure; CO = cardiac output; CVP= central venous pressure; eGFR = estimated glomerular filtration rate; HTx = heart transplantation; ACEi = angiotensin-converting enzyme inhibitor; ARB = angiotensin receptor blocker. MRA = Mineralocorticoid receptor antagonist. Values are corrected for age and gender.

Table 5. Cox regression analysis; independent predictors of mortality at baseline.

Variable	Univariate		Multivariable*	
	HR (95% CI)	P-value	HR (95% CI)	P-value
Heart rate (per 5 bpm)	1.05 (1.03 - 1.08)	< 0.001	1.05 (1.02 – 1.09)	0.003
Sinus Rhythm	0.49 (0.29 – 0.82)	0.006		
Weight (per 5 kg)	0.96 (0.93 – 0.99)	0.006		
Hb (per 1g/dL decrease)	1.10 (1.06 – 1.15)	< 0.001	1.15 (1.08 – 1.23)	< 0.001
Anemia (yes)	2.14 (1.81 – 2.54)	< 0.001		
eGFR (per 10 mL/min/1.73m^2)	0.92 (0.89-0.95)	< 0.001		
CI	0.74 (0.66 – 0.84)	<0.001	0.66 (0.56 – 0.79)	< 0.001
CVP (per 5 mmHg)	1.29 (1.19 – 1.40)	< 0.001		
Medical History				
CAD	1.26 (1.05 -1.50)	0.011	1.31 (1.01 – 1.70)	0.045
Diabetes	1.82 (1.41 – 2.31)	< 0.001	1.97 (1.43 – 2.71)	< 0.001
Reason for Catheterisation				
Congenital Heart Disease	0.37 (0.17 – 0.77)	0.008		
Pre Transplantation	1.50 (1.21 – 1.85)	< 0.001	1.57 (1.10 – 2.54)	0.014
Heart Failure	1.48 (1.22 – 1.80)	< 0.001		
Medication				
Diuretics	1.43 (1.22 – 1.67)	< 0.001	1.26 (0.98 – 1.61)	0.069
ACE/ARB	1.29 (1.10 – 1.52)	0.002		
MRA	1.92 (1.50 - 2.45)	< 0.001	1.48 (1.05 – 2.09)	0.024

*adjusted for all univariate associated variables.

heart rate, diastolic blood pressure and post heart transplantation and CVP were independent predictors of anemia. The relation between CVP and anemia was comparable in patients with a reduced CI and in patients with a normal CI (OR: 1.07 per mm Hg;95%CI 1.02-1.13;p<0.008 vs. OR: 1.13 per mm Hg; 95%CI 1.11-1.19;p<0.0001). Since eGFR influences hemoglobin levels, we performed interaction analysis to study the influence of CVP, CI and eGFR and the presence of anemia. CVP, and eGFR did not have a significant interaction on the presence of anemia (p=0.370) whereas CI and eGFR did have significant interaction on the presence of anemia (p=0.001). The contribution of CVP on the total hemoglobin variance was more pronounced in patients with lower CI compared to patients with a normal CI (table 7).

Prognostic value of anemia, CVP and CI

During a median follow up of 7 years, 707 patients (35%) patients died. In multivariable cox regression analysis, Hb was significantly associated with mortality (HR 1.15 per g/dL decrease;95%CI 1.08-1.23; p<0.001) , whereas CVP was not.(table 5) When entering interaction terms, CVP and hemoglobin had a significant interaction on prognosis (p=0.011). Therefore, to evaluate whether anemia with increased CVP was associated with an adverse outcome, we divided patients into four groups based on the presence

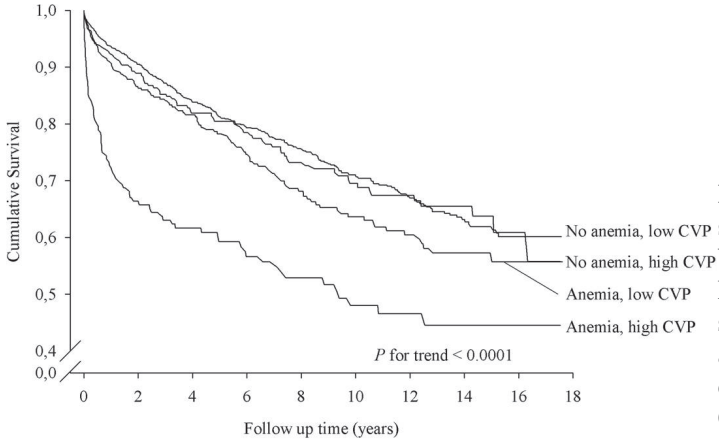

Figure 2. Cumulative survial CVP = Central Venous Pressure. Kaplan Meier plot of cumulative survival categorized according to the presence of anemia and increased CVP.

or absents of anemia and elevated CVP.(table 6) Kaplan Meier survival curves of the four groups are presented in figure 2. Multivariable interaction analysis revealed that anemia with increased CVP was associated with a higher mortality (HR 2.17; 95%CI 1.62-2.90; p<0.0001) than anemia in patients with a normal CVP (HR1.52;95%CI 1.17-1.98; p=0.0002). An elevated CVP was only associated with an impaired outcome in the presence of anemia. Importantly, the increased mortality associated with anemia and CVP was independent of the presence of cardiac dysfunction (figure 3).

Discussion

In the present analysis we show the association of anemia with hemodynamics in a broad spectrum of cardiovascular patients. CI, CVP and SVR are all associated with anemia. Furthermore, anemia in the presence of elevated CVP is associated with a worse prognosis than anemia with normal CVP. Importantly, the association between anemia, CVP and prognosis is independent of cardiac index.

Only limited data exists on the association with anemia and CVP in patients with cardiovascular disease. As mentioned before, experimental studies have shown that

Table 6. Interaction between anemia and CVP on mortality

Variable	Univariate		Multivariable*	
	HR (95% CI)	P-value	HR (95% CI)	P-value
No anemia, low CVP	1.00 (ref)		1.00 (ref)	
No anemia, high CVP	1.04 (0.78 – 1.40)	0.16	0.72 (0.49 – 1.05)	0.12
Anemia, low CVP	1.26 (1.01 – 1.57)	< 0.001	1.51 (1.13 – 2.01)	0.005
Anemia, high CVP	2.56 (1.98 – 3.32)	< 0.001	2.09 (1.48 – 2.96)	< 0.001

*adjusted for all univariate associated variables
CVP = central venous pressure

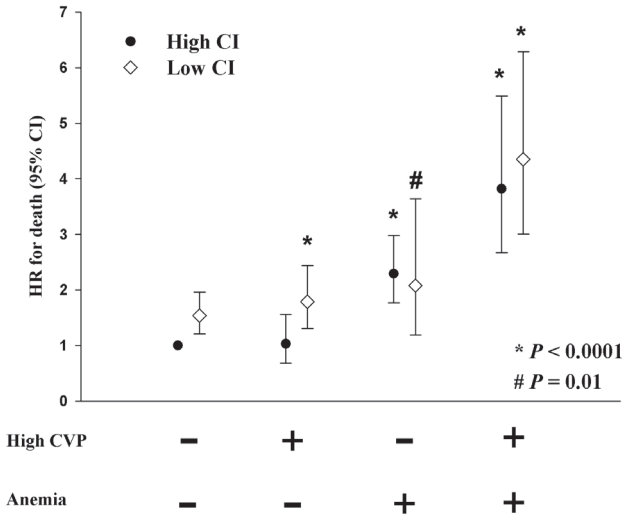

Figure 3. Schematic presentation of survival in different groups. CVP = Central Venous Pressure. CI = Cardiac Index. + = present. - = absent. Data are presented as HR ± 95% CI categorized according to presence of increased CVP and anemia.

anemia lowers blood viscosity, decreasing peripheral vascular resistance and increasing cardiac output, sympathetic tone, neurohumoral activation and heart rate.[23] In our study in human subjects we can confirm these findings that SVR is lower in patients with anemia. In acute moments of isovolumic hemodilution the cardiac output can be increased up to 150% of normal in healthy volunteers.[24] Experimental models of chronic severe anemia have shown that the increase in CO is mediated mainly by an increased SV.[25] In our analysis we observed that, anemic patients have indeed higher cardiac output although this is mainly mediated by an increase in HR. We did not observe differences in stroke volume between anemic and non-anemic patients. This might be explained by the fact that our cohort contains cardiovascular patients with myocardial dysfunction due to different etiologies. These patients might not have the functional capacity to increase SV and rely on HR response to adjust cardiac output.

Perhaps the most intriguing finding of our analysis is the interaction between CI, CVP and anemia. Anemia in patients with cardiac dysfunction (defined as a CI < 2.5 L/m^2) and increased CVP may be the result from congestion. Anemia itself can cause fluid retention through vasodilation which in turn may lead to neurohormonal activation and water and salt retention. However this mechanism probably only plays an important role in patients with severe anemia (Hb<10 g/dL).[26] Since only 6% of our patients had hemoglobin levels below 10 g/dL, it seems more plausible that congestion itself resulted in anemia instead of the opposite.

Table 7. Mulivariable regression analysis for hemoglobin stratified by cardiac index.

Variable	Cardiac Index ≤ 2.5		Cardiac Index >2.5	
	Beta	P -value	Beta	P -value
Age	-0.047	0.4072	-0.014	0.7020
Gender	0.213	0.0003	0.247	< 0.0 001
eGFR	0.159	0.0058	0.235	< 0.0001
CVP	-0.334	0.0001	-0.069	0.0350

eGFR; estimated glomerular filtration rate. CVP; central venous pressure.

In heart failure patients, anemia is commonly observed. In a large meta-analysis we previously showed that one third of the HF patients were anemic.[2] Studies focusing on the etiology of anemia in these patients found that increased extracellular volume was associated with anemia.[27,28] For instance in a study by Westenbrink et al, extracellular volume was measured in chronic HF patients using 125I-Iotholamate. Although patients did not have clinical symptoms of HF, the increased extracellular volume was independently associated with hemoglobin levels.[27,28] Furthermore, much as clinically used jugular vein distention is a poor indicator of extracellular volume or CVP, CVP and extracellular volume are correlated.[26] In our cohort, an elevated CVP was also observed in anemic patients with a normal CI which may indicate that other etiologies with a preserved CI could play a role, for example heart failure with a preserved ejection fraction, isolated right sided heart failure or non-cardiac etiologies including nephrotic syndrome. The contribution of CVP to the total variance of Hb was less in patients with a preserved cardiac function (i.e. CI ≥ 2.5 L/m^2) compared to patients with a low CI. This might suggest that especially in patients with an impaired CI, hemodilution may play a role in the etiology of anemia.

The kidneys play a major role in the etiology of anemia.[29,30] Through production of erythropoietin they are the main stimuli for erythropoiesis. Our current analysis shows indeed that renal function is an important predictor of anemia. One might speculate that the correlation between hemodynamics and anemia can be explained by hypoperfusion of the kidney related to lower perfusion pressure as a result of elevated CVP and lower CI. Indeed, a significant interaction between CI and renal function could be demonstrated on the presence of anemia. This indicates that kidney hypoperfusion could play a role in the presence of anemia. However, a significant interaction with CVP and renal function on the presence of anemia could not be demonstrated, suggesting

that CI is more important for the cardio-renal-anemia axis than CVP.

Regarding survival, CI is an independent predictor of survival in our cohort. We could not demonstrate an independent effect of CVP on outcome in the total cohort. Previously, an elevated CVP has been associated with an impaired outcome, in patients with advanced heart failure, congenital heart disease, lung transplantation and end stage renal failure.[31-34] However these studies did not take hemoglobin levels into account. We found that CVP is only associated with an increased mortality in the presence of anemia, independent of CI and renal function. This additional risk of death when an elevated CVP exists in conjunction with anemia has not previously been described, but may be explained by the strong prognostic value of hemoglobin on outcome and the correlation between CVP and hemoglobin.
There are several limitations to our study. Due to its retrospective design, the etiology of anemia could not be assessed in detail. Therefore, our study merely indicates that there is an association between hemodynamics, anemia and prognosis and we cannot determine a causal relation. It should also mentioned that CVP has a high inter-individual variability and reflects an intra thoracic measurement that can be influenced by numerous factors other than plasma volume, for instance dietary intake and kidney dysfunction.[35] Furthermore, our study included patients with congenital heart disease. As a plethora of congenital heart diseases exists, these could also include patients with Eisenmenger syndrome. These would thus bias our results. As the fraction of these patients in our analysis is only marginal (<5%) and our aim was to study anemia in a general cardiovascular population, we included these patients in our analysis.

In conclusion, anemia is common in cardiovascular patients and is associated with an increased CVP and CI. Patients with both elevated CVP and anemia have the worst prognosis, independent of cardiac index and renal function.

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Chapter 3

Inflammation and anemia in a broad spectrum of patients with heart failure

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Abstract

Aims

Anemia in heart failure (HF) is associated with a poor prognosis. Although inflammation is assumed to be an important cause of anemia the association between anemia and inflammatory markers in patients with HF has not been well established.

Methods

We used data from a multicenter randomized clinical trial in which patients were eligible if > 18 years and admitted for HF (NYHA II-IV). In a subset of 326 patients, hemoglobin, hematocrit, high sensitive C-Reactive Protein (hsCRP), Interleukin-(IL) 6, soluble Tumour Necrosis Factor Receptor (sTNFR)-1, and Erythropoietin (Epo), were measured at discharge and the primary endpoint was all-cause mortality. Follow up was 18 months.

Results

Anemia (Hb < 13 g/dL (men) and < 12 g/dL (women)) was present in 40% (130/326) of the study population. Median levels of IL-6, hsCRP and sTNFR-1 were significantly higher in anemic patients compared to non-anemic patients. Logistic regression demonstrated that each increase of hsCRP values (odds ratio 1.58 per SD log hsCRP; 95%CI: 1.09-2.29; p=0.016) and each increase of sTNFR-1 values (odds ratio 1.62 per SD log sTNFR-1; 95%CI: 1.24-2.11; p<0.001) were independently associated with anemia. Erythropoietin (EPO) (HR 1.31 per log EPO; 95%CI: 1.01-1.69; p=0.041) and sTNFR-1 (HR 1.47 per log sTNFR-1; 95%CI: 1.16-1.86; p=0.001) levels were independently associated with outcome.

Conclusion

Anemia is present in 40% of patients hospitalized for heart failure and is independently associated with inflammation.

Introduction

Anemia is a frequent co-morbidity in patients with heart failure (HF) and its presence is associated with an impaired prognosis.[1] Recent studies have shown that causes for anemia in HF are multifactorial.[1-6] Several mechanisms may contribute to lower hemoglobin (Hb) levels[7], such as an inadequate production of erythropoietin (Epo) due to chronic kidney failure[2, 8], haematinic abnormalities[9], use of medication[10] and bone marrow dysfunction[11]. Elevated levels of cytokines may also play a role in the anemia observed in patients with HF.[12] It has been shown that cytokines have a damaging effect on Epo producing cells in the kidney, thereby causing a reduction of the renal secretion of Epo and as a consequence, an inadequate Epo response for the degree of anemia.[13,,14] Indeed, experimental studies showed that cytokines impair hematopoiesis in CHF following myocardial infarction.[15] In addition, cytokines directly affect bone marrow function, by impairing proliferation and differentiation of erythroid precursor cells.[16, 17]

Inflammation has already been proposed as an important cause of anemia in HF.[4, 18, 19]. It has been demonstrated that inflammatory cytokines have a significant prognostic value in patients with HF.[20-22] However, only few studies have looked into the association between different pro-inflammatory cytokines and its effect on anemia in HF. Therefore, we used a well defined cohort of HF patients and studied the association between anaemia, EPO levels and several inflammatory cytokines.

Methods

Patient Selection

The COACH (the Coordinating study evaluating Outcomes of Advising and Counseling in HF) study was a multicenter, randomized nurse-led trial to evaluate the effect of two interventions (basic support vs. intensive support) compared with care as usual in HF patients. Background, rationale and results of the COACH trial (NCT98675639) are published elsewhere.[23,24] Patients were eligible for trial inclusion if they were at least 18 years of age, had a hospital admission for HF (New York Heart Association class II-IV), and had evidence of structural underlying heart disease. Participants were randomized during hospitalization for HF. The primary outcome was a composite endpoint of hospitalization for HF or all cause mortality. A total of 1023 patients were included in COACH and were followed for up to 18 months. Additional blood samples were collected at baseline (hospital discharge) for measurement of Epo and cytokine levels. The 326 patients included in this substudy had levels of inflammatory factors and he-

moglobin available at baseline. Patients did not belong to a specific intervention group. This study complies with the Declaration of Helsinki, local medical ethics committees approved the study, and all patients provided written informed consent.

Laboratory assessments

The abbreviated Modification of Diet in Renal Disease equation was used to estimate the glomerular filtration rate (eGFR).[25] Anemia was defined according to the World Health Organization (WHO) criteria as a hemoglobin level < 13.0 g/dL in men and a hemoglobin level < 12.0 g/dL in women.[26] Plasma Epo levels were measured using the IMMULITE EPO assay (Diagnostic Products Corporation, Los Angeles, California).[27]

Plasma cytokine assays

Blood samples were drawn and EDTA plasma was immediately stored at -80º Celcius until analysis was be performed. Levels of Interleukin (IL)-6, soluble tumour necrosis factor receptor 1 (sTNFR-1), and high sensitive C-Reactive Protein (hsCRP) were measured in plasma samples using SearchLight® Proteome Arrays (Aushon BioSystems, Billerica, MA). The SearchLight Proteome Array is a quantitative multiplexed sandwich ELISA containing up to 12 different capture antibodies spotted on the bottom of a 96-well polystyrene microtiter plate. Each antibody captures a specific protein present in the standards and samples added to the plate. The bound proteins are then detected with a biotinylated detection antibody, followed by the addition of streptavidin-horseradish peroxidase (HRP) and lastly, a chemiluminescent substrate. The luminescent signal produced from the HRP-catalyzed oxidation of the substrate is measured by imaging the plate using the SearchLight Imaging System which is a cooled charge-coupled device (CCD) camera. The data is then analysed using SearchLight Array Analyst software. The same spiked controls are distributed across individual plates and the amount of luminescent signal produced is proportional to the amount of each protein present in the original standard or sample. Concentrations are extrapolated off a standard curve.

Statistical Analysis

Results are presented as mean ± standard deviation (SD) when normally distributed and as median and interquartile range (IQR) when non-normally distributed. Comparisons of differences between groups were made by unpaired Student's t-test, χ2-test or Mann-Whitney U testing when appropriate. The relation between known predictors of anemia or hemoglobin and cytokines was assessed with standard linear or logistic regression analysis using standardized values. Because of skewed distribution, creati-

nine, NT-proBNP, Epo, sTNFR-1 and hsCRP were transformed to natural logarithmic transformation before entering them in a regression model. The variables age, creatinine, NT-proBNP, sTNFR-1, hsCRP , IL-6 and epo were assessed for their univariate association with hemoglobin or anemia. Other variables did not last trough the univariate screen. Variables that showed a significant ($p<0.10$) univariate association were manually entered in a stepwise backward multivariate model based on the strength of their univariate association. Thus before progressing to the next step of the model the variable with the highest p-value was excluded from the model. Additional bootstrap analysis was performed to measure accuracy of the estimated model. Variables that showed a significant univariate association ($p<0.10$), were randomly selected for their multivariate association with either anemia or Hb. This cycle was repeated 1000 times and variables selected more than 700 times were assumed to be accurate selected variables.

Univariate Cox proportional hazard regression models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI) associated with baseline hsCRP, IL-6, TNFR-1, anemia and death from any cause. Multivariate Cox regression analysis was performed to analyze predictors of all-cause mortality. Univariable significant ($p<0.10$) predictors (age, creatinine, sTNFR-1, hsCRP, Epo and Hemoglobin) were entered in a multivariate backward stepwise model based on their strength of univariate association. Additionaly, interaction analysis of anemia, hb and cytokines was performed.

Data were complete for hemoglobin and eGFR measurements. The following variables had data missing: IL-6 (patients with missing data n=24), sTNFR-1 (n=35), hsCRP (n=22), and NT-proBNP (n=16). All missing data were excluded for analyses. All tests were 2-tailed and a p-value<0.05 was considered statistically significant. All analyses were performed with STATA/IC for Windows version 11.0 (StataCorp LT, College Station, TX).

Results

Patient characteristics

Baseline characteristics of all 326 patients with values of both inflammatory factors and hemoglobin available at baseline are presented in Table 1. Baseline characteristics from the patients in COACH did not differ from the 326 subjects included in this subanalysis (supplementary file, table 1). Mean age was 71±11 years, 61% was male and mean left ventricular ejection fraction (LVEF) was 0.35±0.14. At discharge, 40% of patients (82 men, 48 women) were anemic. These patients were significantly older, had worse

Table 1. Baseline characteristics of the total population

Variable	Total	Non-Anemic	Anemic	p-value
N	326	196	130	
Age (years)*	71 ± 11	70 ± 12	73 ± 11	0.021
Female gender	127 (39%)	79 (40%)	48 (37%)	0.540
LVEF (%)*	35 ± 14	34 ± 15	36 ± 14	0.112
NYHA class III+IV (n/%)	159 (49%)	98 (50%)	61 (47%)	0.570
BMI kg/m^2*	26.8 ± 5.5	27.0 ± 5.2	26.4 ± 5.9	0.262
Diabetes II (n/%)	62 (19%)	32 (16%)	30 (23%)	0.128
History of hypertension	138 (42%)	83 (42%)	55 (42%)	0.994
Medication at discharge (n/%)				
ACE inhibitor/ARB	257 (79%)	166 (85%)	91 (70%)	0.001
Beta blocker	221 (68%)	138 (70%)	83 (64%)	0.214
Diuretics	308 (95%)	188 (96%)	120 (92%)	0.162
Routine laboratory at discharge				
Hemoglobin (g/dL)*	13.2 ± 2.0	14.4 ± 1.4	11.2 ± 1.0	N.a.
Hematocrit (L/L)*	0.40 ± 0.06	0.44 ± 0.04	0.34 ± 0.03	<0.001
Creatinine (µmol/L)†	115 (90 -146)	109 (89 -136)	131 (97 -160)	0.001
Blood urea nitrogen (mg/dL)*	11.2 ± 5.8	10.6 ±5.8	12.1± 5.9	0.987
eGFR (ml/min/1.73m^2)†	53.5 (39.4-67.3)	57.3 (42.1-71.1)	46.4 (33.8-63.8)	0.002
NT-proBNP (pmol/dL)†	2532 (1327-5552)	2227 (1113-4430)	3802 (1671-7507	<0.001
Epo (U/L)†	10.8 (5.6-17.4)	8.2 (4.3 -16.3)	13.2 (9.4 -20.2)	<0.001
Inflammatory markers at discharge				
IL-6 (pg/ml)†	12.0 (7.1-25.5)	10.6 (5.9-23.2)	14.8 (9.3 -31.0)	0.002
sTNFR-1 (mg/ml)†	3.7 (2.1 -4.4)	3.3 (2.0 -3.9)	4.27 (2.4 -5,4)	0.002
hsCRP (mg/L)†	2.3 (0.9 -5.2)	2.0 (0.8 -4.6)	3.4 (1.3 -6.6)	0.001

The presence of anemia was defined at baseline (hospital discharge); LVEF= Left ventricular ejection fraction; NYHA=New York Heart Association; BMI= Body mass index; ACE=angiotensin converting enzyme; ARB=Angiotensin receptor blocker; eGFR=estimated glomerular filtration rate; NT-proBNP=N-Terminal pro B-type Natriuretic Peptide; Epo=erythropoietin; IL=Interleukin; sTNFR-1= soluble tumour necrosis factor receptor 1; hsCRP= high sensitive C-Reactive Protein; *mean ± sd; †median/IQR; P-value: anemic vs non-anemic .

renal function, and higher NT-proBNP levels (Table 1). During 18 months of follow up, 49 (38%) patients died in the anemic cohort, compared to 46 (24%) in the non-anemic group (p=0.006 between anemic and non-anemic patients).

Circulating levels of cytokines

Baseline levels of IL-6, hsCRP and sTNFR-1 were significantly higher in anemic patients compared to non-anemic patients (Table 1). Logistic regression analysis was performed to study the relation between anemia and inflammatory cytokines(Table 2). Besides Epo (OR 1.47 per SD log ; 95CI:1.11-1.93; p=0.006), anemia was independently associated with sTNFR-1 (OR 1.62 per SD log ; 95%CI:1.24-2.11; p<0.001) and with hsCRP (OR 1.58 per SD log 95%CI:1.09-2.29; p=0.016). Bootstrap analysis revealed these parameters were selected accurately (supplementary file, table 2). Also in multivariate linear regression analysis, sTNFR-1 (Beta 0.470 per log sTNFR-1; 95%CI: 0.291-0.680; p<0.001) and hsCRP (Beta 0.308 per SD log sTNFR-1; 95%CI: 0.023-0.550; p=0.033) were independent predictors of hemoglobin levels.

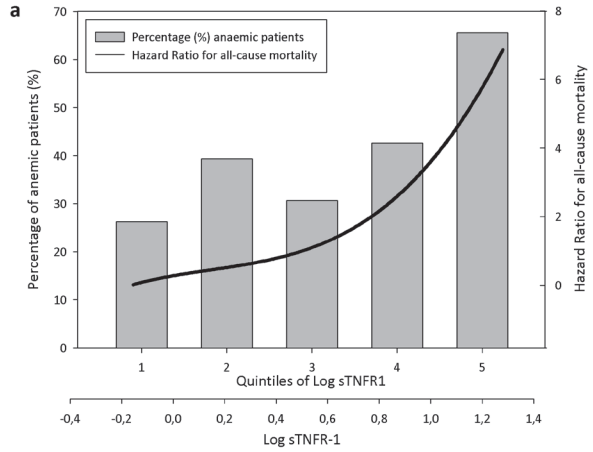
Prognostic value of cytokines

As presented in Figure 1, increased levels of sTNFR-1, hsCRP and IL-6 are univariably associated with increased mortality. In a multivariable model, both Epo (HR 1.31 per log EPO; 95%CI:1.01-1.69; p=0.041), and sTFR-1 (HR 1.47 per log sTNFR-1; 95%CI:1.16-1.86; p=0.001) were independent predictors of mortality, whereas hemoglobin was only univariate a predictor of mortality (table 3). A statistically significant interaction between the presence of anemia and the prognostic value of inflammatory cytokines could not be demonstrated.

Discussion

The present study shows an independent association between inflammatory factors and anemia in HF patients. Levels of hsCRP, sTNFR-1 and IL-6 are significantly higher in anemic HF patients compared to those without anemia. Second, increased levels of hsCRP and sTNFR-1 independently predict anemia and mortality in HF patients. sTNFR-1 was associated with an impaired survival, whereas anaemia was not. In our study, anemia is present in 40% of patients, which is high compared with other HF studies in which anemia rates from 9.3% up to 42.6% are reported. [28-33]Since patients in the present study were included while hospitalised for HF, the higher prevalence of anemia might be explained by hemodilution.[5, 34] However, laboratory measurements were taken just before discharge, when patients were already stabilized. Another explanation for the presence of anemia in HF is an impaired renal function.[35]Due to a decreased cardiac output, renal blood flow is decreased. Low renal perfusion may eventually lead to renal ischemia and finally this will lead to anemia due to decreased

A



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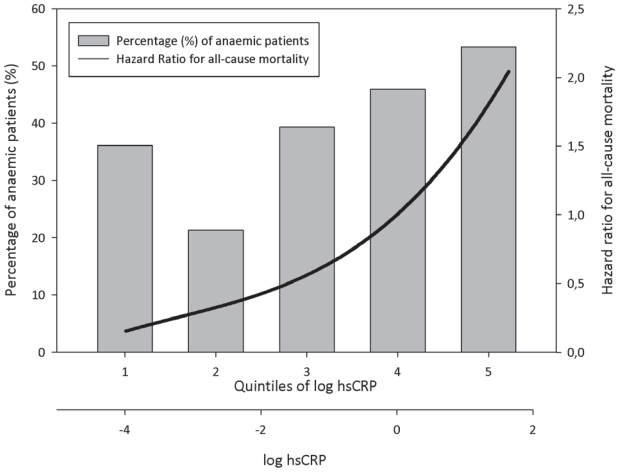

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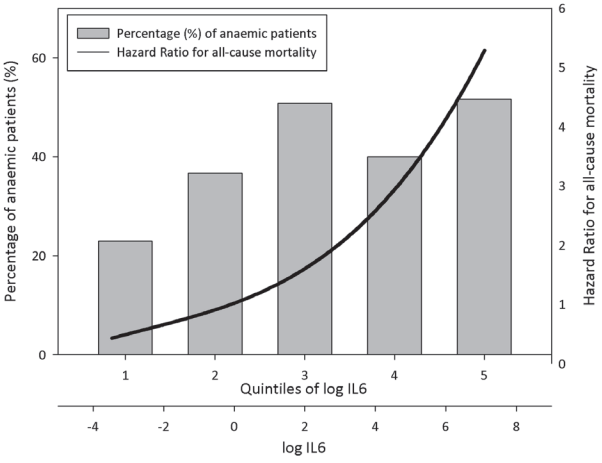

Figure 1. Combined bar/line graphs representing levels of sTNFR-1 (Figure 1a), hsCRP (Figure 1b) or IL-6 (Figure 1c), the percentage of anemic patients per quintile of sTNFR-1 (Figure 1a), hsCRP(-Figure 1b) or IL-6 (figure 1c) and the univariate Hazard Ratio for all cause mortality per log sTNFR-1 (Figure 1a), log hsCRP (Figure 2b) or log IL-6 (figure1c).

Epo production. Other causes of decreased hemoglobin levels are a disturbed iron homeostasis and elevated levels of circulating cytokines.[17]

Iron is crucial for the formation of hemoglobin and inflammation leads to a reduced absorption. Recent data indeed suggest that intravenous iron (bypassing intestinal uptake) improves cardiac function.[36] Furthermore, as we previously have shown, in patients with anemia the bone marrow becomes less sensitive to erythropoietin (EPO). Although EPO levels are elevated in HF, it might be insufficient to overcome the reduced sensitivity of the bone marrow which might be related to inflammation.[3]

The association between anemia and inflammation in HF has been previously studied. Opasich et al. reported that patients with anemia and chronic HF had higher cytokine levels and that hemoglobin values were inversely related to IL-6 levels.[4] In a study by Dunlay et al., TNF-α levels were measured in 486 patients with HF. They established an association with higher TNF-α and anemia.[18] In another study, higher fibrinogen levels were found to be an independent predictor of anemia.[19] However, these studies did not investigate a variety of inflammatory factors in a multivariate analysis nor did they study the prognostic value of elevated cytokines in anemic HF patients. In the present study, we investigated several inflammatory factors which are known to influence haemoglobin levels negatively. For example, TNF-α and IL-6 are involved in processes concerning iron-homeostasis.[17] Iron homeostatis is disturbed in heart failure patients and indeed recent data shows improved cardiac function after intravenous iron suppletion. TNF-α induces the synthesis of ferritin, thereby stimulating iron storage in macro-

Table 2. Logistic regression analysis; independent predictors of anemia at baseline.

Variable	Univariable		Multivariable	
	OR (95% CI)	p - value	OR (95% CI)	p - value
Age	1.31 (1.04 - 1.65)	0.022		
Creatinine*	1.52 (1.20 - 1.92)	<0.00 1		
NT - proBNP*	1.53 (1.20 - 2.00)	0.001		
sTNFR - 1*	1.79 (1.39 - 2.29)	<0.001	1.62 (1.24 - 2.11)	<0.001
hsCRP*	1.46 (1.10 - 1.93)	0.008	1.58 (1.09 - 2.29)	0.016
Epo*	1.77 (1.38 - 2.27)	<0.001	1.47 (1.11 - 1.93)	0.006
IL6	1.35 (1.10- 1.65)	0.004		

All covariates were measured at baseline (hospital discharge); *the odds ratios for creatinine hsCRP, Epo NT-proBNP and STNFR-1 refer to the odds per SD increase of the log transformed variable; NT-proBNP=N-Terminal pro B-type Natriuretic Peptide; sTNFR-1= soluble tumour necrosis factor receptor 1; hsCRP=high sensitive C-reactive protein; Epo=Erythropoietin; IL6=Interleukin 6.

Table 3. Hazard Ratios (95% CI) for all cause mortality

Variable	Univariable HR (95% CI)	p-value	Multivariable HR (95% CI)	p-value
Age	1.66 (1.32-2.10)	<0.001	1.57 (1.20-2.05)	0.001
Creatinine*	1.58 (1.33-1.89)	<0.001		
NT-proBNP*	2.15 (1.71-2.71)	<0.001	1.82 (1.36-2.45)	<0.001
sTNFR -1*	1.96 (1.61-2.40)	<0.001	1.47 (1.16-1.86)	0.001
hsCRP*	1.55 (1.16-2.07)	0.003		
IL6	1.27 (1.07-1.49)	0.005		
Epo*	1.53 (1.25-1.86)	<0.001	1.31 (1.01-1.69)	0.041
Hb*	0.77(0.61-0.91)	0.003		

All covariates were measured at baseline (hospital discharge); *the hazard ratios for sTNFR-1, creatinine, hsCRP, Epo, Hb and NT-proBNP refer to increase of the log transformed variable; sTNFR-1=soluble Tumour Necrosis Factor Receptor 1; NT-proBNP=N-Terminal pro B-type Natriuretic Peptide; IL6=Interleukin 6.

phages and it also blocks the release of iron from these cells. Although evidence exists that TNF-α is responsible for negative hematopoietic effects and apoptosis, the effects are mediated by its receptor, sTNFR-1.[37] Moreover, this TNF-α receptor has been proven to be prognostic in chronic HF patients, although its relation with anemia in these patients has not been described before.[38] IL-6 stimulates hepcidin expression, a protein that inhibits duodenal iron absorption.

Pro-inflammatory cytokines are also involved in the suppression of erythropoiesis in the bone marrow. TNF-α is associated with impairment of the proliferation and differentiation of erythroid progenitor cells, possibly by the induction of apoptotic cell death via sTNFR-1 and by stimulating free radical formation which has a toxic effect on erythroid precursor cells.[16, 17, 39, 40] More Epo is therefore needed in order to reach the same level of erythrocytes, but despite the higher Epo levels often seen in anemic HF patients, they are still insufficient for the hemoglobin level.[3, 13] An inadequate production of Epo might also be caused by increased levels of TNF-α, which have a direct negative effect on Epo gene transcription and can also damage Epo-producing cells in the kidney.[17] We recently described that 80% of anemic HF patients had Epo levels lower than expected on the basis of their hemoglobin compared to 30% in the non-anemic group.[13] However, we acknowledge that both pro-inflammatory cytokines and renal function may influence Epo production. Since pro-inflammatory cytokines have multiple effects the exact contribution of each factor is difficult to discern. Both anaemia and inflammatory cytokines have a deleterious effect on prognosis, although a significant interaction could not be demonstrated. We therefore cannot postulate a causal relationship between anemia

and inflammatory cytokines.

Several limitations of the present study have to be mentioned. This is a retrospective analysis of a large cohort of HF patients of whom 40% are anemic. Unfortunately, hematinic parameters and hemodilution are not measured to further elucidate the cause of anemia in these patients. Furthermore, we could not show a significant interaction between inflammatory cytokines and anemia, Inflammatory factors are only assessed at hospital discharge and no further information is available regarding other underlying diseases which are known to increase cytokines (e.g. malignancies, infections or autoimmune diseases).

In summary, anemia is present in 40% of patients hospitalized for HF and is independently associated with inflammation. Inflammation and EPO levels are independently associated with outcome.

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Supplemental information

Table 1. Comparison original cohort versus substudy cohort.

Variable	Original cohort	Substudy cohort
N	1023	326
Age (years)*	71 ± 11	71 ± 11
Female gender	389 (38%)	127 (39%)
LVEF (%)*	34 ± 14	35 ± 14
NYHA class III+IV (n/%)	522(51 %)	159 (49%)
BMI kg/m ^{2} *	27 ± 5	27 ± 6
Diabetes II (n/%)	187 (18 %)	62 (19%)
History of hypertension	439 (43%)	138 (42%)
Medication at discharge(n/%)		
ACE inhibitor/ARB	847(83%)	257 (79%)
Beta blocker	677(66%)	221 (68%)
Diuretics	980(95%)	308 (95%)
Routine laboratory at discharge		
Hemoglobin (g/dL)*	13.2 ± 1.9	13.2 ± 2.0
NT -proBNP (pmol/dL) †	2521 (1293 – 5548)	2532 (1327 -5552)

The presence of anemia was defined at baseline (hospital discharge); LVEF= Left ventricular ejection fraction; NYHA=New York Heart Association; BMI= Body mass index; ACE=angiotensin converting enzyme; ARB=Angiotensin receptor blocker; NT-proBNP=N-Terminal pro B-type Natriuretic Peptide; *mean ± sd; †median/IQR.

Table 2. Bootstrap analysis referring table 1

Variable	Bootstrap analysis Times selected
Age	186
Creatinine*	298
NT -proBNP*	117
sTNFR -1*	761
hsCRP*	788
Epo*	889

Table 3. Logistic regression analysis; independent predictors of heamoglobin at baseline.

Variable	Univariable		Multivariable	
	Beta (95%CI)	p-value	Beta (95% CI)	p-value
Age	0.450 (0.236 -0.664)	<0.001	0.275 (0.066–0.485)	0.010
Creatinine*	0.351 (0.134 -0.569)	<0.001		
Diabetes II	0.255 (0.474 -0.035)	0.023		
NT-proBNP*	0.467 (0.246 –0.688)	0.002		
sTNFR-1*	0.670 (0.456 –0.884)	<0.001	0.470 (0.291-0.680)	<0.001
hsCRP*	O.359 (0.129 –O.580)	0.001	0.306 (0.023–0.550)	0.033
Epo*	O.720 (O.512 –0.927)	<0.001	0.507 (0.291 –0.724)	<0.001

All covariates were measured at baseline (hospital discharge); the beta's for hsCRP, Epo and STNFR-1 refer to the beta per SD increase of the log transformed variable; NTproBNP=N-Terminal pro B-type Natriuretic Peptide; sTNFR-1= soluble tumour necrosis factor receptor 1; hsCRP=high sensitive C-reactive protein; Epo=Erythropoietin.

Chapter 4

Impact of postoperative anemia on cardiovascular outcome after Coronary artery bypass graft surgery; insights from the IMAGINE trial

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Abstract

Objective

To investigate the association between sustained postoperative anaemia and outcome after coronary artery bypass graft (CABG) surgery.

Design

Retrospective analysis of the IMAGINE trial, which tested the effect of the ACE inhibitor quinapril on cardiovascular events after CABG.

Setting

Thoracic surgery clinic/outpatient department. Patients 2553 stable patients with left ventricular ejection fraction >40% 2-7 days after scheduled CABG.

Interventions

Randomisation to quinapril or placebo. Main outcome measures Cox regression analysis for the association between postoperative anaemia and cardiovascular events and the effect of quinapril on the incidence of anaemia.

Results

Postoperative anaemia was sustained for >50 days in 44% of patients. Sustained postoperative anaemia was associated with an increased incidence of cardiovascular events during the first 3 months (adjusted HR (adjHR) 1.77, 95% CI 1.10 to 2.85, p=0.012) and during the maximum follow-up of 43 months (adjHR 1.37, 95% CI 1.14 to 1.65, p=0.008). When haemoglobin (Hb) was considered as a continuous variable, every 1 mg/dl decrease in Hb was associated with a 13% increase in cardiovascular events (adjHR 0.87, 95% CI 0.81 to 0.95, p=0.003) and a 22% increase in all-cause mortality (adjHR 0.78, 95% CI 0.60 to 0.99, P=0.034). Quinapril was associated with a slower postoperative recovery of Hb levels and a higher incidence of cardiovascular events in patients with anaemia (adjHR 1.60, 95% CI 1.1 to 2.4, p=0.024).

Conclusions

Postoperative anaemia is common, frequently persists for months after CABG surgery and is associated with an impaired outcome. In patients with anaemia, ACE inhibitors slowed recovery from postoperative anaemia and increased the incidence of cardiovascular events after CABG.

Introduction

Anaemia is a well-established predictor of impaired cardiovascular outcome in patients with coronary artery disease,[1] acute coronary syndromes,[2] chronic heart failure[3] as well as in the general population.[4] In addition, the presence of anaemia before cardiac and non-cardiac surgery and during extracorporeal bypass predicts postoperative cardiovascular events.[5-7] The consistent association between anaemia and cardiovascular events suggests that the severity and duration of postoperative anaemia should be limited in patients undergoing coronary artery bypass graft (CABG) surgery. There are, however, several reasons to suspect a high incidence of anaemia after CABG that may often persist for months after discharge. First, blood loss during CABG surgery is common owing to the nature and extent of the surgery and also because of required heparinisation and additional acquired defects in haemostasis during on-pump procedures. Second, during on-pump procedures anaemia is often induced in patients by mild euvolaemic haemodilution. Third, blood transfusions are deliberately avoided because they have been linked to impaired survival after CABG.[8-10] If a substantial proportion of patients remain anaemic for an extended period after CABG, this would suggest that patients are subjected to an established cardiovascular risk factor by an intervention that is intended to reduce that risk. Although the impact of preoperative anaemia and perioperative transfusions have been addressed extensively, the incidence of sustained postoperative anaemia and its impact on the outcome after CABG is unclear. We hypothesised that sustained postoperative anaemia after CABG surgery is common and associated with an increased incidence of cardiovascular events. In order to test our hypothesis we used the contemporary IMAGINE (Ischaemia Management with Acupril post bypass Graft via Inhibition of thecoNverting Enzyme) trial database of 2553 patients with preserved left ventricular function undergoing CABG.[11,12] Because ACE inhibitors have the capacity to reduce erythropoiesis activity and might increase the incidence and duration of sustained postoperative anaemia,[13,14] we also evaluated the effects of quinapril on postoperative anaemia.

Methods

Design

The design of the IMAGINE trial has been described in detail previously,[11] as well as the results of the main study.[12] In brief, the IMAGINE study was a double-blind placebo-controlled parallel-group randomised multicentre international trial conducted in

patients who underwent CABG surgery between November 1999 and September 2004. The main goal of the study was to test whether early initiation of ACE inhibitor therapy (initiated within the hospital phase) after CABG would reduce the rate of cardiovascular events in patients at relative low risk. The research protocol was approved by the ethics committees of all participating institutions and all patients provided written informed consent.

Patients

Patients were screened for eligibility within 4 weeks of surgery or following surgery and randomised within 7 days after CABG, except for patients included in France (N=235, 9.1%) where randomisation occurred within 10 days after CABG. Treatment consisted of the ACE inhibitor quinapril, with forced uptitration to 40 mg daily within 4 months if tolerated, or matching placebo. The final sample size of the IMAGINE study was 2553 patients.

Anaemia

Anaemia was defined according to the WHO criteria (haemoglobin (Hb) <13.0 g/dl in men and Hb <12.0 g/dl in women). We explored the temporal characteristics of anaemia in the entire IMAGINE population. For outcome analysis, patients were classified into two groups: those with sustained anaemia at 50 days after randomisation (anaemic group) and those in whom Hb levels had recovered to normal values during that period (non-anaemic group). Because the definition of anaemia represents a relatively arbitrary Hb cut-off, we also evaluated the effects of Hb as a continuous variable at 50 days after randomisation. Because our analysis was focused on sustained postoperative anaemia, we excluded patients with normal Hb levels at randomisation and patients without documented Hb levels at 50 days (leaving 2400 subjects).

Endpoints

We used the same primary and secondary endpoints as in the IMAGINE trial, including an additional composite endpoint of major adverse cardiac events (MACE), and we also considered all individual endpoints separately. The primary endpoint was a composite of cardiovascular death or resuscitated cardiac arrest, non-fatal myocardial infarction, coronary revascularisation, unstable angina requiring hospitalisation, documented angina not requiring hospitalisation, stroke and congestive heart failure (CHF) requiring hospitalisation. The pre-specified secondary endpoint was a composite of the primary endpoint with the addition of transient ischaemic attack and any other cardiovascular

event requiring hospitalisation. MACE was defined as cardiovascular death or resuscitated cardiac arrest, acute coronary syndromes, coronary revascularisation and CHF requiring hospitalisation. Because we expected that postoperative anaemia would predominantly affect the early postoperative phase, we also separately evaluated the effect of sustained postoperative anaemia on the primary IMAGINE endpoint during and after the first 3 months of the study.

Statistical analysis

Data are shown as mean ± SD when normally distributed, as median (IQR) in cases of skewed distribution and as frequencies and percentages for categorical variables. Differences in variables between groups were compared with the Student t test, the ManneWhitney U test, χ^2 test or Fisher exact test, where appropriate. Time to the first event was calculated from 50 days onward, except for the analysis of the first 3 months where time to first event was calculated from randomisation. Temporal changes in Hb levels and the incidence of anaemia were compared using ANCOVA for repeated measurements. Differences between the anaemic and non-anaemic groups, use of Hb as a continuous variable and the effect of quinapril treatment were estimated as a HR with associated adjusted two-sided 95% CI from a Cox proportional hazards regression model that included the effects of age, gender, treatment assignment, country, transfusions, number of days after CABG surgery, cardiac medications, baseline Hb levels, left ventricular ejection fraction (LVEF), smoking status, systolic and diastolic blood pressure and creatinine values at baseline or at 50 days after randomisation, history of hypertension/diabetes/percutaneous coronary interventions/myocardial infarction/previous CABG surgery/peripheral vascular disease and stroke, vessel disease, number of distal anastomoses, completeness of revascularization (defined as complete when all vessels >1 mm with a stenosis >70% were bypassed) and beating heart (off pump) surgery.

Cumulative event rates were calculated by the Kaplan-Meier method and displayed graphically. Differences in the incidence of the component endpoints within or after the first 3 months were assessed by univariate logistic regression analysis. All statistical analyses were performed using SPSS Version 17.0.

Results

Incidence of anaemia

Patients were randomised an average of 462 days after CABG surgery. During randomisation, 2400 patients (94%) were anaemic according to the WHO criteria for anaemia

and 444 (19%) had Hb values <9 mg/dl. However, only 11 (3%) of the patients with Hb levels <9 mg/dl received a blood transfusion during the first 10 days after randomisation. Hb levels increased steadily during the first year (figure 1), at which point 163 (8%) of the patients were still anaemic (figure 1). Hb levels and the proportion of anaemic subjects remained constant after 1 year (figure 1). Throughout the study period anaemia was reported as an adverse event in 140 patients (6%). In 30 patients (1.2%), diagnostic or therapeutic measures were taken in response to anaemia. Blood transfusions were given to 21 patients (0.8%) during the entire study period. Of the 13 patients who received a blood transfusion within the first 50 days after randomisation, nine had recovered from anaemia at 50 days.

Demographic characteristics of the study population

The characteristics of the study group are given in table 1. At the visit 50 days after randomisation, 967 (44%) of the 2180 patients with anaemia at baseline and available Hb levels at 50 days were still anaemic. Patients with sustained postoperative anaemia were significantly older, had a higher incidence of previous CABG surgery and were more often current smokers. Anaemic patients had a higher incidence of anaemia at randomisation, lower red blood cell, white blood cell and platelet counts and significantly higher creatinine values. Preoperative LVEF was comparable between anaemic and non-anaemic patients with CHF, but systolic and diastolic blood pressures were

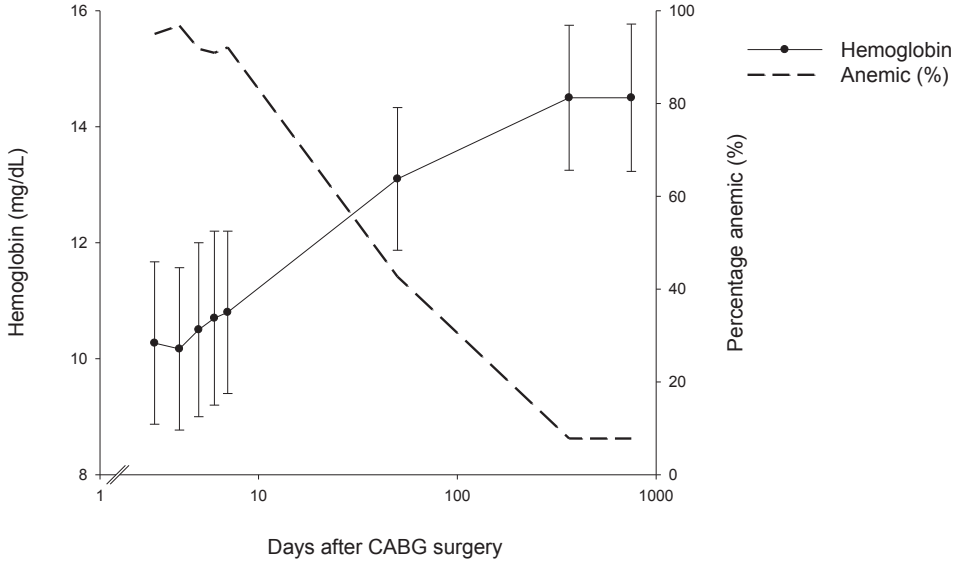

Figure 1. Temporal changes of hemoglobin levels and the percentage of patients with anemia after coronary artery bypass graft surgery.

Table 1. Demographics of the study population at 50 days after randomization according to the presence of sustained postoperative anemia

Variable	Non-anemic (n=1327)	Anemic (n=988)	P
Age	59 ± 10	63 ± 9	>0.0001
Female, n (% of patients)	164 (12)	117 (12)	0.748
White, n (% of patients)	1278 (96)	949 (96)	0.741
Acupril group, n (% of patients)	637 (48)	505 (51)	0.110
Anemia at baseline, n (% of patients)	1213 (92)	967 (98)	>0.0001
Days after CABG surgery, Mean ± SD	24 (2)	24 (2)	0.661
Medical History, n (% of patients)			
Previous MI	529 (40)	382 (39)	0.576
Previous Stroke	17 (1)	33 (3)	0.001
Previous CABG	22 (2)	35(4)	0.004
Previous PCI	233 (18)	177(18)	0.869
Diabetes	118 (9)	105 (11)	0.176
History of hypertension	598 (45)	476 (48)	0.152
Current smoker	288 (22)	176 (18)	0.021
Laboratory values, Mean ± SD			
Hemoglobin (mg/dL)	14 ± 0.8	12 ± 0.8	-
RBC ($x10^{12}$/L)	4.7 ± 035	4.1 ± 0.36	>0.0001
White blood count ($x10^{9}$/L)	7.2 ± 2	7.0 ± 2	0.064
Platelets ($x10^{9}$/ L)	292 ± 83	270 ± 68	>0.0001
Total cholesterol (mmol/L)	4.9 ± 1	4.8 ± 1	0.372
LDL cholesterol (mmol/L)	2.9 ± 1	23 ± 52	0.263
HDL cholesterol (mmol/L)	1.1 ± 0.3	1.1 ± 0.4	0.811
Creatinine (μmol/L)	88 ± 17	90 ± 19	>0.0001
Hemodynamic measurements, Mean ± SD			
LVEF (%)	60 ± 96	60 ± 96	0.341
Systolic blood pressure (mmHg)	128 ± 18	125 ± 18	>0.0001
Diastolic blood pressure (mmHg)	78 ± 10	74 ± 10	>0.0001
Operative characteristics			
Beating heart surgery, n (% of patients)	264 (20)	171 (17)	0.119
Number of distal anastomosis, Mean±SD	3.2 ± 1.2	3.3 ± 1.1	0.023
Vessel disease, Mean ± SD	2.5 ± 0.7	2.6 ± 0.6	0.003
Complete revascularization, n (% of patients)	723 (55)	523 (53)	0.448
Baseline medications, n (% of patients)	1044 (79)	778 (79)	1.000
Beta blocker	486 (37)	353 (36)	0.622
Calcium channel inhibitor	37 (2.8)	31 (3.1)	0.621
Angiotensin receptor blocker	969 (73)	751 (76)	0.113
Platelet inhibitor	854 (64)	633 (64)	0.895
Statin	125 (94)	84 (85)	0.464
Diuretic			

SD, standard deviation; MI, myocardial infarction; CABG, coronary artery bypass graft surgery; PCI, percutaneous coronary intervention; RBC, red blood cell count; LDL, low density lipoprotein; HDL, High density lipoprotein; LVEF, left ventricular ejection fraction.

significantly lower in the patients with anaemia. Anaemic patients more often had multivessel disease. There was no difference in the number of distal anastomoses or incomplete revascularisations between the groups. Finally, baseline medication was comparable between anaemic and non-anaemic patients.

Events

The median follow-up time was 1082 days (IQR 160e1323). Univariate differences in events according to the presence of sustained postoperative anaemia are shown in table 2. Anaemia resulted in a 77% increase in the primary endpoint within the first 3 months after randomisation (adjusted HR (adjHR) 1.77, 95% CI 1.10 to 2.85, p=0.012, table 2, figure 2). After 3 months the incidence of the different endpoints was comparable between groups (data not shown). Anaemia did not affect the incidence of the primary endpoint after 50 days. However, the incidence of the secondary IMAGINE endpoint and MACE were both increased by 40% in patients with anaemia (table 3, figure 3). When analysed as a continuous variable, lower Hb levels were also associated with an impaired outcome after CABG (table 3). In fact, every decrease in Hb of 1 mg/dl was associated with a 13% increase in the incidence of the secondary endpoint and MACE and a 22% increase in all cause death or resuscitated cardiac arrest (table 3). The primary endpoint was not associated with continuous Hb levels.

Effect of quinapril on postoperative anaemia

Hb levels were identical at baseline in patients randomized to quinapril and placebo (10.43 ± 61.45 vs 10.41 ± 61.45 mg/dl, p=0.783). Similarly, the proportion of anaemic patients was similar at baseline in the quinapril and placebo groups (1198 (94%) vs 1202 (95%), p=0.796). The proportion of patients with diabetes was comparable between the quinapril and placebo groups and background medications were also comparable (data not shown). After randomisation, recovery of Hb levels was slower in patients randomised to quinapril than in those receiving placebo and remained lower in the quinapril group throughout the follow-up period (figure 4A). Similarly, a higher proportion of patients remained anaemic in the quinapril group than in the placebo group throughout the follow-up period (figure 4A).

Effect of quinapril on outcome in anaemic patients

In the subgroup of patients who were anaemic at randomisation, 99 patients (4.1%) experienced a primary endpoint during the first 3 months, 61 (5.1%) in the quinapril group and 38 (3.2%) in the placebo group (HR 1.62, 95% CI 1.1 to 2.4, figure 4B). A sec-

Table 2. Univariate and multivariate hazard ratios for primary and secondary endpoints

	Persistent anemia		Hemoglobin continuous	
Endpoints	Unadjusted HR (95% CI)	Adjusted †‡ HR(95% CI)	Unadjusted HR (95% CI)	Adjusted † HR (95% CI)
Primary composite	1.19 (0.95-1.50)	1.16 (0.91-1.49)	0.89 (0.81- 0.97)*	0.89 (0.81-0.98)#
- First 3 months	1.94 (1.04-3.02)*	1.78 (1.10 - 2.87)*	0.78 (0.66-0.92)*	0.83 (0.68-0.99)*
- After 3 months	1.00 (0.77-1.32)	1.00,(0.75-1.35)	0.92 (0.82-1.02)	0.92 (0.81-1.04)#
Secondary composite	1.30 (1.08-1.57)*	1.32 (1.08-1.61)#	0.87 (0.81-0.94)§	0.88 (0.81 -0.95)

HR, hazard ratio; CI, confidence intervals; †; Adjusted for, age, gender, treatment assignment, country, days after CABG-surgery, transfusions, left ventricular ejection fraction, systolic and diastolic blood pressure, creatinine, history of hypertension / diabetes / percutaneous coronary interventions / myocardial infarction / previous CABG surgery / peripheral vascular disease / stroke, number of distal anastomosis,completeness of revascularization and beating heart (off pump) surgery, ‡; also adjusted for baseline hemoglobin levels. *, P<0.05; #, P<0.005; §, P<0.0005

ondary event was experienced in 102 (43%) patients, 62 (5.2%) in the quinapril group and 40 (3.3%) in the placebo group (HR 1.57, 95% CI 1.1 to 2.3, p=0.026). Quinapril significantly increased the incidence of the primary and secondary endpoints during the first 3 months. It did not affect the incidence of the primary and secondary endpoints after 3 months, nor did it affect the outcome when the entire study period was considered (data not shown).

Discussion

In this study we show for the first time that sustained postoperative anaemia after CABG surgery is associated with an impaired outcome. Although some degree of mild to moderate anaemia might be expected in the immediate postoperative period, we show that postoperative anaemia is the rule rather than the exception, is frequently severe and often persists for months in a substantial proportion of patients. Moreover, anaemia generally seems to be regarded as a benign condition, as it was sparsely reported as an adverse event and only very few patients were diagnosed or treated in our contemporary population. In this study we show that sustained postoperative anaemia may not be benign, but is rather associated with an impaired outcome even in low-risk patients. In addition, we show that the initiation of an ACE inhibitor in the early postoperative phase after CABG slows postoperative recovery of Hb levels and is associated with an increase in early cardiovascular events. We therefore propose that more aggressive measures to prevent or limit postoperative anaemia and perioperative discontinuation of ACE inhibitors could improve the outcome after CABG.

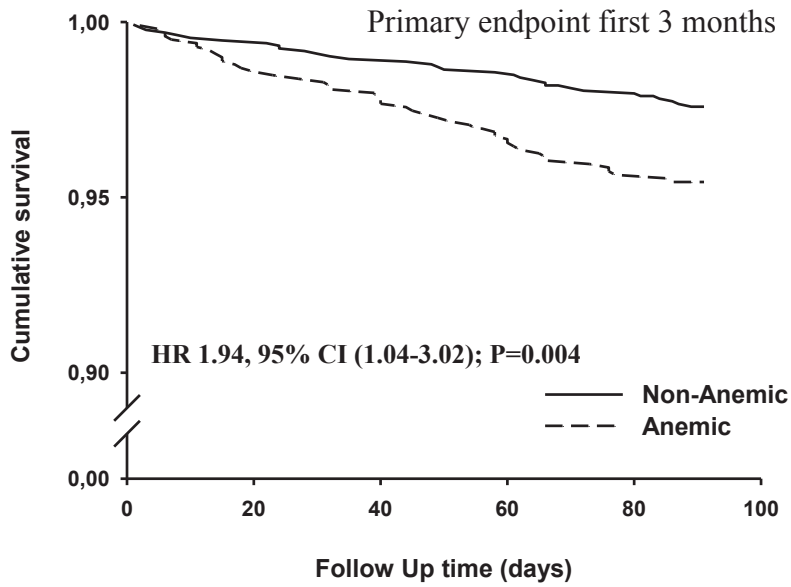

Figure 2. Kaplan-Meier analysis of event free survival of the primary endpoint during the first 3 months according to the presence or absence of sustained postoperative anemia. Primary composite endpoint of the IMAGINE trial comprising time to first occurrence of cardiovascular death or resuscitated cardiac arrest, nonfatal myocardial infarction, coronary revascularization, unstable angina that required hospitalization, documented angina that did not require hospitalization, stroke and congestive heart failure that required hospitalization. HR, Hazard Ratio; 95% CI, 95 percent confidence interval.

Incidence, aetiology and impact of sustained postoperative anaemia

The few studies that have addressed the impact of postoperative anaemia on outcome in patients undergoing CABG surgery were restricted to the immediate postoperative ICU period.[10,15,16] One of the most surprising findings of our study is that it frequently takes months to recover from anaemia after CABG, even in a lowrisk population with few comorbidities. Similar to other cardiac populations, the aetiology of anaemia after CABG surgery is likely to be multifactorial including perioperative blood loss, haemodilution, bone marrow depression and persisting preoperative causes for anaemia such as haematinic deficiencies or renal dysfunction.[17-19] Despite its high incidence, anaemia was documented as an adverse event in only 6%, and diagnosed and treated in only 1% of anaemic patients. These findings strongly suggest that anaemia is not considered as an important comorbidity after CABG. However, we clearly show that sustained anaemia is associated with a marked increase in cardiovascular events. In our opinion, postoperative anaemia should therefore not be regarded as a benign condition and increased awareness of the importance of anaemia after cardiac surgery is thus warranted. Effect

Table 3. Incidence of the primary and secondary endpoints and their components during the first 3 months after randomization

Variable	Non-anemic (n=1327)	Anemic (n=988)	P
Primary composite endpoint	33 (2.5)	47 (4.8)	0.004
Cardiovascular death or resuscitated cardiac arrest	1 (0.1)	1 (0.1)	1.000
Non-fatal myocardial infarction	3 (0.2)	3 (0.3)	0.705
Unstable angina requiring hospitalization	7 (0.5)	11 (1.1)	0.150
Documented angina not requiring hospitalization	20 (1.5)	15 (1.5)	1.000
Coronary revascularisation	0 (0)	1 (0.1)	0.427
Stroke or TIA	0 (0)	2 (0.2)	0.182
CHF requiring hospitalization	2 (0.2)	11 (1.1)	0.003
Any other cardiovascular event	0 (0)	3 (0.3)	0.078
Cardiac ischemia composite endpoint*	31 (2.3)	31 (3.4)	0.244
Cardiac composite endpoint #	33 (2.5)	42 (4.5)	0.024

*Composite of cardiovascular death or resuscitated cardiac arrest, nonfatal myocardial infarction, coronary revascularization, unstable angina that required hospitalization, documented angina that did not require hospitalization. # composite of the above with the addition of congestive heart failure requiring hospitalization; TIA, transient ischemic cerebrovascular attack; CHF, congestive heart failure

of ACE inhibitors on postoperative anaemia ACE inhibitors reduce erythropoiesis in patients with heart failure through increased levels of N-acetyl-seryl-aspartyl-lysylproline, a haematopoiesis inhibitor exclusively depredated by ACE.[13] Moreover, a small Italian study showed that enalapril slowed postoperative recovery of Hb levels when given early after CABG.[20] In our analysis, the early initiation of quinapril after CABG slowed recovery of Hb levels and was also associated with early cardiovascular events. Due to the expanding indications for ACE inhibitors in patients with coronary artery disease, a large number of patients scheduled for CABG surgery will be treated with these drugs. Importantly, a recent retrospective study of 10 000 British patients undergoing CABG surgery showed that ACE inhibitors are often continued during surgery or re-initiated early after CABG. Furthermore, the authors showed that preoperative treatment with an ACE inhibitor was linked to increased mortality.[21] Our finding that quinapril increased early cardiovascular events when given to anaemic patients in the early postoperative phase suggests that the detrimental effects of perioperative ACE inhibitor therapy can in part be explained by the delayed recovery of Hb levels.

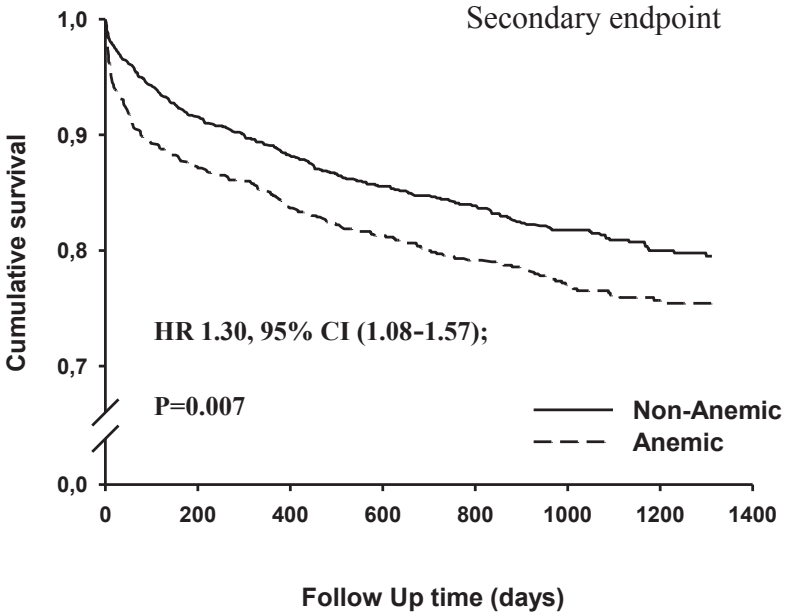

Figure 3. Kaplan-Meier analysis of event free survival of the secondary endpoint during the maximal follow up of 43 months according to the presence or absence of sustained postoperative anemia. Secondary composite endpoint of the IMAGINE trial comprising time to first occurrence of cardiovascular death or resuscitated cardiac arrest, nonfatal myocardial infarction, coronary revascularization, unstable angina that required hospitalization, documented angina that did not require hospitalization, stroke or TIA, congestive heart failure that required hospitalization and all other cardiovascular hospitalizations. HR, Hazard Ratio; 95% CI, 95 percent confidence interval.

Transfusions

Another important observation in our analysis was that blood transfusions were only sparsely administered, despite the high incidence of severe anaemia. While this approach is in accordance with current guidelines, it may in part explain the high incidence of sustained postoperative anaemia.[17] Koch et al recently showed that the use of older blood products is associated with mortality after CABG, suggesting that the risks of transfusions are more related to the quality of the blood than the transfusion itself.[22] Together these findings suggest that the balance between risk and benefit of transfusions after CABG should be reconsidered. Our study does not, however, address the safety and efficacy of blood transfusions after CABG surgery and we cannot recommend more lenient transfusions based on our retrospective study. A prospective evaluation is urgently awaited.[23]

Limitations

Ideally, we would have liked to have data on the exact duration of postoperative anae-

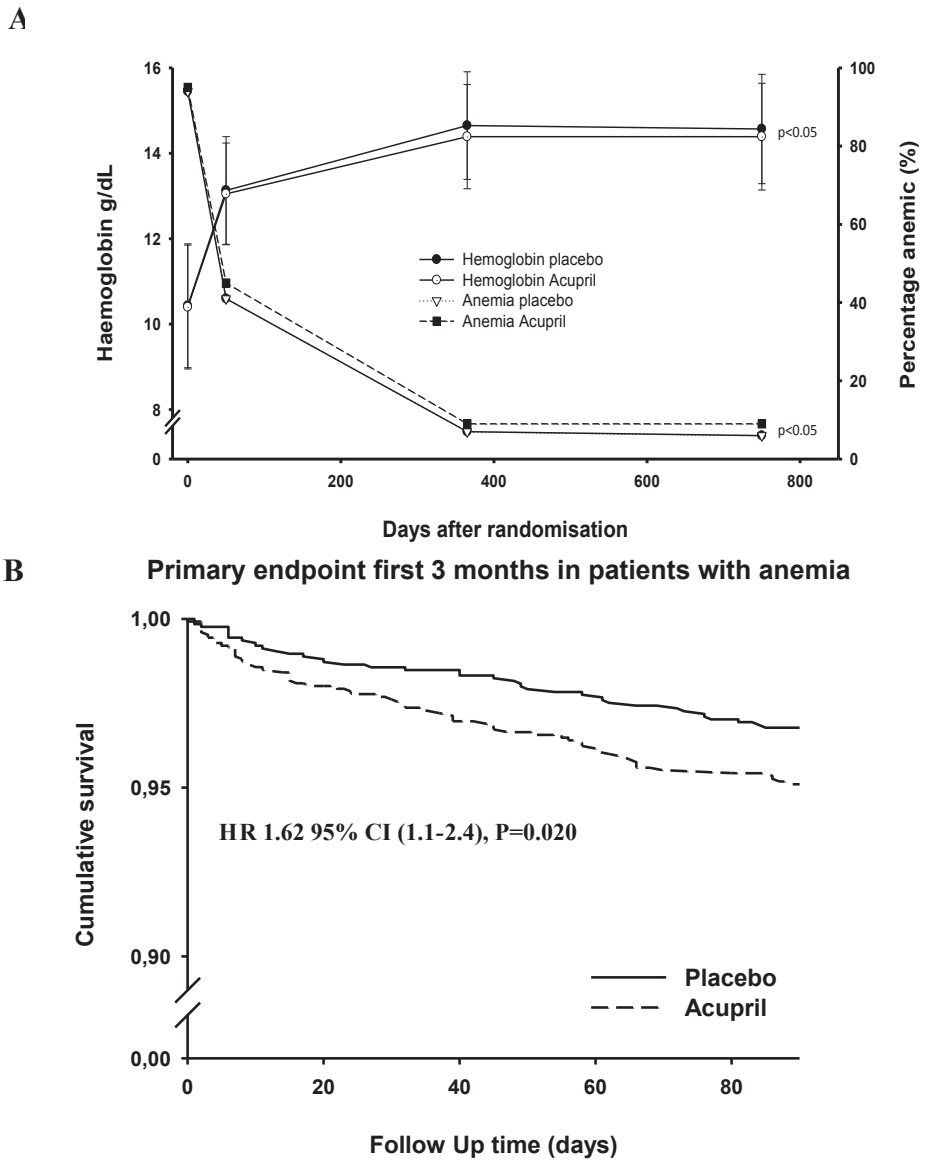

Figure 4. Effect of Acupril on postoperative recovery of hemoglobin levels and the effect of Acupril on survival in patients with anemia. A. Temporal changes of hemoglobin levels and percentage of patients with anemia after coronary artery bypass graft surgery, in patients randomized to Acupril or placebo. B. Kaplan-Meier analysis of event free survival of the primary endpoint during the first 3 months according to treatment groups, namely Acupril or placebo. Primary composite endpoint of the IMAGINE trial comprised time to first occurrence of cardiovascular death or resuscitated cardiac arrest, nonfatal myocardial infarction, coronary revascularization, unstable angina that required hospitalization, documented angina that did not require hospitalization, stroke and congestive heart failure that required hospitalization. HR, Hazard Ratio; 95% CI, 95 percent confidence interval.

mia in all patients. Unfortunately, the frequency of Hb measurements in the IMAGINE protocol was fairly sparse and the first post-discharge Hb value was measured 50 days after randomisation. Early events that occurred while a patient was anaemic but in whom Hb had recovered at the 50-day post-randomisation visit were therefore attributed to the non-anaemic group. Therefore, the number of early events associated with the presence of anaemia is potentially underestimated in our analysis. Similarly, we cannot exclude the possibility that events that occurred before 50 days affected the duration of postoperative anaemia. This limitation should be borne in mind when interpreting the analysis of the early events. In addition, defining groups at 50 days after randomization excludes patients who died before 50 days. The IMAGINE study involved a relatively healthy low-risk population, so the deleterious effects of anaemia might be different in the general CABG population. Finally, despite the use of extensive multivariable adjustments, we cannot be certain that the relation between anaemia and events is causal since we employed a retrospective analysis of prospectively-collected data. Alternatively, sustained postoperative anaemia might be a marker for a high-risk subgroup of patients. Future studies are needed to further define the impact of postoperative anaemia on outcome after CABG surgery.

Clinical implications

Our study shows that the duration of postoperative anaemia is an important determinant of outcome after CABG. We therefore propose more aggressive measures to limit the severity and duration of postoperative anaemia. This could, for instance, be achieved by increased utilisation of contemporary strategies to limit the duration and severity of postoperative anaemia, such as minimal invasive surgery, autologous blood transfusions, thrombostatic drugs and perhaps erythropoiesis-stimulating proteins.[17] It also seems logical to evaluate Hb levels early after discharge in order to identify and treat patients with sustained postoperative anaemia in an early phase. Furthermore, our data suggest that perioperative discontinuation of ACE inhibitors could prevent sustained postoperative anaemia. Prospective studies are required to determine whether these measures will reduce postoperative anaemia and improve the outcome after CABG surgery.

Conclusions

Postoperative anaemia is common, frequently persists for months after CABG surgery and is associated with an impaired cardiovascular outcome. ACE inhibitors slow postoperative recovery of Hb levels after CABG and increase cardiovascular events in pa-

tients with anaemia.

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Chapter 5

Impaired erythropoiesis after coronary artery bypass grafting is associated with an increased inflammatory response

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Dirk J. van Veldhuisen
B. Daan Westenbrink
Peter van der Meer

Submitted

Abstract

Background:

Sustained postoperative anemia after Coronary Artery Bypass Graft (CABG) is associated with an impaired outcome. The reason that anemia sustains in these patients is unknown. We hypothesized that the degree of post-operative inflammation is inversely related to erythropoiesis efficiency after CABG.

Methods:

Consecutive patients that underwent elective CABG surgery at the University Medical Center Groningen between 2009 and 2011 were identified and their data were extracted from electronic Databases. A normal reticulocyte response was defined as an increase in reticulocyte count (RC) compared to pre-operative values. The relationship between changes in RC,hemoglobin (Hb) and inflammation was assessed with multivariable logistic regression analysis.

Results

A total of 1147 patients were included in the analysis. Hb decreased from 13.9±1.58 g/dL to 10.5±1.60 g/dL 3 days after surgery, whereas RC increased from 12 (IQR 10-15) ‰ to 17 (IQR 14-22) ‰. The increase in RC after CABG was inversely related to changes Hb, C-reactive protein (CRP) and creatinine. An insufficient reticulocyte response was present in 126 (11%) of patients. Changes from baseline in Hb and CRP were independently associated with an insufficient reticulocyte response.

Conclusion

Impaired erytropoiesis after CABG surgery is associated with an increased inflammatory response.

Background

Anemia is common in patients with various cardiovascular diseases, including heart failure, myocardial infarction and stable coronary disease.[1-3]

The presence of anemia in these patients is associated with increased morbidity and mortality, suggesting that anemia could represent a nodal point for intervention.

Despite all efforts to prevent blood loss, more than 90% of patients develop anemia after CABG.[4,5] Cardiovascular patients are thus subjected to an established cardiovascular risk factor by an intervention that is intended to reduce that risk. Indeed, we recently demonstrated that sustained postoperative anemia is associated with an impaired outcome after CABG.[6] These findings suggest that more aggressive measures to prevent or limit postoperative anemia could improve outcome after CABG.

The factors responsible for the impaired recovery from anemia are, however, unknown. In heart failure patients bone marrow suppression has been identified as a central mechanism driving the increased susceptibility to anemia.[7,8] Inflammatory cytokines are potent suppressors of erythropoiesis and inflammation has been associated with anemia through mechanisms involving bone marrow suppression.[9] Because major surgeries such as CABG inflict a systemic inflammatory response syndrome, we hypothesised that the degree of post-operative inflammation is inversely related to erythropoiesis efficiency after CABG. For this purpose, we analysed the association between post-operative reticulocyte response and inflammation following CABG surgery.

Methods

In the current study we retrospectively analysed all consecutive patients that underwent CABG surgery at the University Medical Center Groningen (UMCG) between 2008 and 2009. Data was extracted from the patients file and only unique cases were used. The study was performed following the UMCG research code and approved by the local medical ethical committee.

Laboratory assessment

With every routine Hemoglobin (Hb) measurement a reticulocyte count (RC) was also performed in the same sample. Reticulocyte count is expressed as promille (‰) of circulating erythrocytes. Routine C-reactive protein (CRP) and creatinine concentrations were also determined in the central laboratory using lithium heparin tubes. A reticulocyte response was defined as an increase in RC at day 3 compared to pre-operative values.

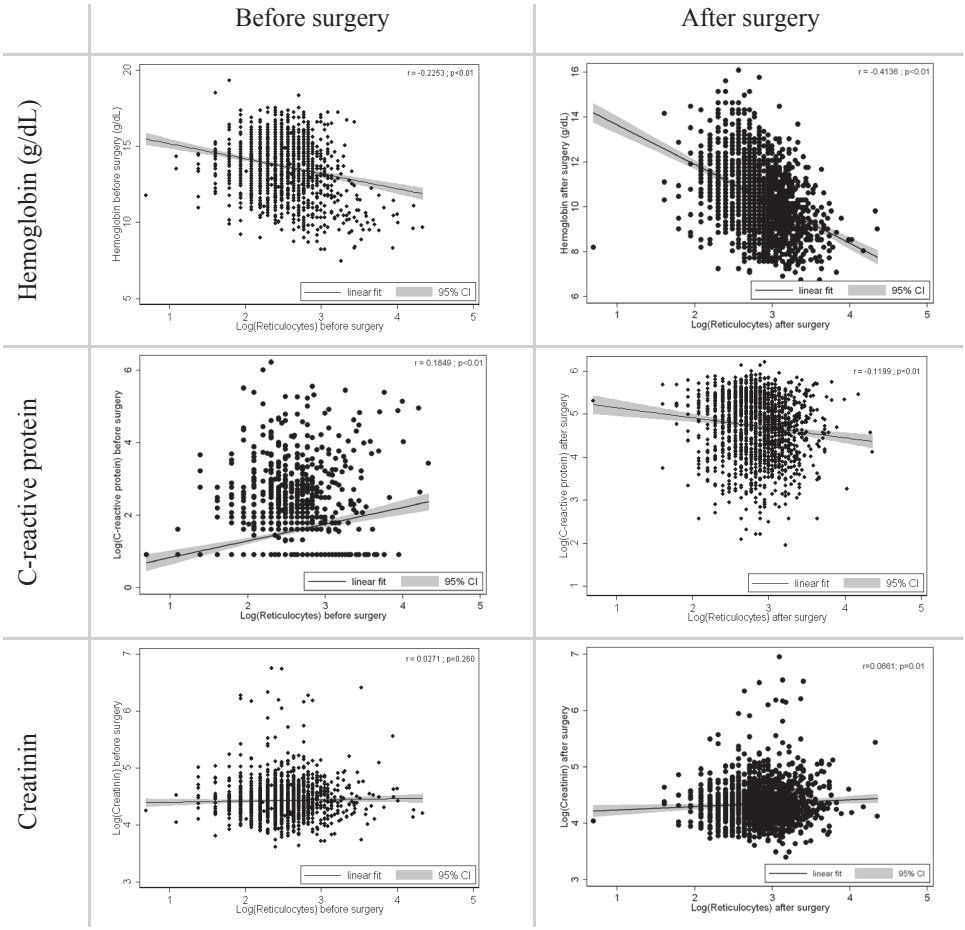

Figure 1. Scatter plots of hemoblobin, C-reative protein and creatinin in relation to reticulocytes both before and after surgery. Scatter plots according to variable and time before or after surgery. R=regression coefficient, CI: coinfidence interval.

Statistical Analysis

Results are presented as mean ± standard deviation (SD) when normally distributed, as median and interquartile range (IQR) when non-normally distributed and as frequencies and percentages for categorical variables. Comparisons of differences between groups were made by unpaired Student's t-test, Kruskall Wallis test when appropriate. The relation between reticulocyte response and Hb, creatinine, CRP, sex and age was assessed with logistic regression analysis using standardized values. Variables that showed a significant ($p<0.10$) univariate association were entered in a multivariable regression model using backward selection. In this model, variables that did not retain significance were subsequently removed from the model based on the strength of their association.

Results

Study cohort

A total of 1147 patients were studied. Mean age was 66 (IQR; 58-74) years and the majority of the patients (66%) were male. Hb dropped from 13.9±1.58 g/dL before surgery to 10.5±1.60 g/dL 3 days after surgery. Pre-operative anemia was present in 206 (18%) patients, whereas 1113 (97%) of the patients were anemic 3 days after surgery.

Reticulocyte response

Average RC increased from 12 (IQR 10-15) ‰ before surgery to 17 (IQR 14-22) ‰ at 3 days after surgery. In 126 patients there was no detectable increase in RC. Characteristics stratified according to the presence or absence of a reticulocyte response at day 3 are depicted in table 1. Patients with no increase in RC were more often male, were younger, and had higher hemoglobin levels, 3 days after surgery. Furthermore, CRP levels were higher both before and after surgery.

Univariate predictors of erythropoiesis

Figure 1 depicts the relation between reticulocytes and Hb, creatinine or CRP before and after surgery. As expected, there was a significant correlation between Hb and RC before and after surgery. Before surgery there was a small yet significant correlation between RC and CRP. In contrast, there was strong inverse correlation between CRP

Table 1. Baseline characteristics by reticulocyte response

Variable	No increase (n=126)	increase (n=1021)	P value
Age (years)	59 ± 14	63 ± 14	<0.01
Female (%)	18%	34%	<0.01
Reticulocytes (‰)			
Before surgery	13 (11-17)	11 (9-14)	<0.01
Day 3	13 (10 – 17)	17 (14-21)	<0.01
Hemoglobin (g/dL)			
Before surgery	14 (12 – 15)	14 (13 -15)	0.94
Day 3	12 (10- 13)	10 (9-11)	<0.01
CRP (mg/L)			
Before surgery	2.5 (2.5 – 10)	2.5 (2.5 – 6)	0.01
Day 3	167 (100 – 238)	121 (64 – 194)	<0.01
Creatinin e (µmol/L)			
Before surgery	81 (72-92)	81 (70 – 92)	0.27
Day 3	75 (65-86)	74 (63-88)	0.24

and RC 3 days after surgery. Accordingly, there was a significant univariate association between changes from baseline in Hb and RC, CRP and RC and creatinine and RC.

Multivariable predictors or erythropoiesis

Logistic regression analysis was used to identify predictors of an impaired reticulocyte response (table 2). Sex, the post-operative drop in Hb and post operative increase in CRP levels were independently associated with an insufficient reticulocyte response.

Discussion

Post-operative anemia is virtually inevitable after major surgery such as CABG. Nevertheless, the severity and duration of anemia is inversely related to outcome. Recovery from anemia relies heavily on efficient erythropoiesis in the bone marrow, suggesting that erythropoiesis is suppressed in patients with sustained post-operative anemia. In the current analysis we show, for the first time, that the severity of the systemic inflammatory response after CABG is inversely related to the efficiency of erythropoiesis. These findings suggest that suppression of erythropoiesis by inflammatory cytokines is may underlie sustained post-operative anemia.

The link between inflammation and anemia

The association between inflammation and anemia is well described and appears to be a major underlying etiology for anemia in various chronic diseases, including heart failure.[10] While inflammation can cause anemia through several complementary mechanisms including hypoferremia due to increased iron cell uptake and iron retention.[10,11] suppression of erythropoiesis is perhaps the most well described.[12,13] Inflammatory cytokines such as TNF-α, interferon γ, interleukin 1 and interleukin 6 inhibit proliferation of erythroid progenitior cells. Also, these cytokines reduce erythropoietin production and decrease the sensitivity of the bone marrow to erythropoietin.[9,14,15] Acute systemic inflammatory responses also occur during surgery, trauma, and sepsis.[16] Erythropoiesis efficiency has also been shown to be associated with a systemic inflammatory response syndrome in these patients.[17] To the best of our knowledge, this is the first study to show that inflammation after CABG surgery is associated with suppression of erythropoiesis.

Clinical implications

Chronic inflammatory diseases are common in patients scheduled for CABG.[18]As shown, patients with impaired reticulocyte response had increased CRP levels before surgery. Patients with active chronic inflammation therefore probably reflect a high

Table 2. logistic regression analysis for reticulocyte increase at 3 days after surgery

Variable	Univariable		Multivariable	
	Odds ratio (95% CI)	p-value	Odds ratio (95% CI)	p-value
Age	1.23 (1.05 -1.45)	0.01		
Sex	1.49 (1.20 – 1.86)	<0.01	1.37 (1.05 – 1.78)	0.02
Hb change	0.23 (0.17 -0.31)	<0,01	0.25 (0.18 -0.33)	<0.01
Creatinin e change	0.81 (0.62 - 1.03)	0.09		
CRP change	0.66 (0.55 -0.80)	<0.01	0.68 (0.59 – 0.89)	<0.01

risk group that should be intensely monitored to limit the duration of post-operative anemia after CABG surgery. In addition, it would be prudent to avoid erythropoiesis inhibiting factors such as angiotensin convering enzyme (ACE) inhibitors in the early post operative phase.[6]

Limitations

The limitations are that this retrospective analysis is necessarily an observational analysis and that despite all efforts; the potential for confounding can never be fully eliminated. For instance, patients were selected on the basis of reticulocyte and hemoglobin values, which may result in selection bias. Moreover, potentially confounding variables such as transfusion, medication, comorbidities were not available for our multivariable analysis. Our study should thus be regarded as hypothesis generating.

In conclusion, impaired erythropoiesis after CABG surgery is associated with an increased inflammatory response.

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Part 2 | Erythropoietin in cardiovascular disease

Chapter 6

Should erythropoietin treatment in chronic heart failure be hemoglobin targeted?

Editorial to "A meta-analysis of erythropoiesis-stimulating agents in anaemic patients with chronic heart failure"
by Jin et al, Eur J Heart Fail. 2010 Mar;12(3):249-53

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Eur J Heart Fail. 2010 Mar;12(3):215-6

Erythropoeitin (EPO) is traditionally known for its haematopoietic effect on the bone marrow, trough salvaging erythroid precursors from apoptosis. Blunted EPO production can cause anaemia. In chronic heart failure (CHF) anaemia is prevalent and associated with a poor prognosis.[1] Therefore, erythropoiesis stimulating agents (ESA's) have been extensively investigated in a large number of experimental studies, and showed to possess cardioprotective effects as well. In addition, several studies have been performed to evaluate safety, feasibility, and clinical outcome of ESA treatment in patients with heart failure.

In the present issue of the European Journal of Heart Failure, Jin et al. present a meta-analysis on the effects of ESA on clinical outcome in patients with CHF. Seven clinical trials were included of which four evaluated darbepoietin alfa and three investigated other ESA's. ESA treatment was associated with a mean increase in left ventricular ejection fraction of 7.55%. NYHA class improved by 1.2 and KCCQ scores improved by 4.61 points. The authors conclude that the treatment of anaemia with ESA in patients with symptomatic CHF can improve cardiac function, exercise capacity and quality of life.

The current study did not perform an analysis of hospitalization due to heart failure. In an earlier meta-analysis, the risk for hospitalization due to heart failure was proved to be decreased (risk ratio 0.59) in the ESA treated group.[2] Similar to that study, Jin et al. were also unable to establish a significant protective effect of ESA treatment on overall mortality, although a strong trend was observed. The study by Jin et al. is particularly interesting because it strongly suggests that ESA treatment may also improve quality of life.

But how can these potentially beneficial effects of ESA on cardiac function and clinical outcome in heart failure patients be explained?

With the discovery of a functional EPO receptor in tissue other than bone marrow, it was hypothesised that EPO elicits extra-hematopoietic effects as well.[3] This was supported when a functional EPO receptor was discovered in heart tissue.[4] Exogenous stimulation of these receptors in animal models with human recombinant EPO proved to decrease infarct size and ameliorated left ventricular function.[5] These results might be explained by the finding that stimulation of the EPO receptor in the heart increases neovascularisation through increase of vascular endothelial growth factor (VEGF) and endothelial progenitor cells (EPC) homing to the myocardium.[6] In CHF patients myocardial cells grow (hypertrophy) without a concomitant growth of the number of capillaries; subsequently leading to a demand-supply mismatch of oxygen and nutrients.[7] Therefore, especially in chronic heart failure, ESA's might be an attractive approach to improve this demand-supply mismatch.

In addition to delayed neovascularisation after ischemia, EPO exerts antiapoptotic effects as well. Following myocardial infarction, EPO prevents apoptosis of cardiomyocytes and this in turn might decrease permanent damage and loss of function. These findings led to the design of a randomized multicentre study to evaluate whether EPO can attenuate postmyocardial infarction loss of cardiac function.[8]

Interestingly, the beneficial effects of ESA's on myocardial function have been described with doses that did not influence haemoglobin and/or haematocrit.9 In addition, similar cardiac effects were demonstrated with carbamylated erythropoietin (CEPO), a non-erythropoietic derivative of erythropoietin.10 Therefore, the observed beneficial cardiac effects of ESA might not only originate from increased haemoglobin levels, but are rather due to the previously described non-haematopoietic effects of EPO. The definite mechanisms still remain elusive.

Safety-issues

The Trial to Reduce Cardiovascular Events With Aranesp® Therapy (TREAT), performed to evaluate cardiovascular events in chronic kidney disease, showed that treatment with darbepoietin alfa was associated with an increased risk of stroke and more hospitalisations.[9] In a study in patients with stroke, ESA's were associated with an increased incidence of stroke recurrence.[11] Therefore, concerns are raised about the safety of ESA's. It is unclear however whether the safety issues are similar in patients with heart failure.[12] Jin et al. did not draw any conclusion about safety. They do however see a non-significant decrease in mortality in favour of the ESA treated group. Also, in the studies in patient with renal caused anaemia, the major aim was to improve clinical outcome with ESA's through the increase of haemoglobin. In heart failure however, beneficial effects of ESA's are probably caused by its non-haematopoietic effects.[13] Therefore, new agents like CEPO are being developed that could elicit the beneficial protective of effects of EPO, without its undesirable erythropoietic side effects.[14]

Taken together, the study by Jin et al. confirmed that EPO has a large therapeutic potential in patients with heart failure and indicates there is a growing urge for larger studies to be performed. The ongoing Reduction of Events With Darbepoetin Alfa in Heart Failure (RED-HF) trial is a large multicentre, double-blind, randomized, placebo controlled trial, that will hopefully provide a definitive answer to the question whether erythropoietin in anaemic chronic heart failure will decrease mortality and morbidity.[15] It should however be noted that in RED-HF erythropoietin is titrated to attain certain haemoglobin levels. So far about two thirds of the patients are recruited and the expected date of completion is in 2012. With the development of novel agents that have sim-

ilar cardioprotective effects, without increase of haemoglobin, the application of these agents might even be widened to non-anaemic heart failure patients as well.

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Chapter 7

Long term effects of epoetin alfa in patients with ST-Elevation myocardial infarction

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Abstract

Purpose

The HEBE III trial showed that epoetin alfa administration in patients with a first ST-elevation myocardial infarction (STEMI) did not improve left ventricular function at 6 weeks after primary percutaneous coronary intervention (PCI). The long term effects of erythropoiesis- stimulating agents on cardiovascular morbidity and mortality are unknown, therefore we evaluated clinical events at 1 year after PCI.

Methods

A total of 529 patients with a first STEMI and successful primary PCI were randomized to standard optimal medical treatment (N = 266) or an additional bolus of 60,000 IU epoetin alfa administered intravenously (N = 263) within 3 hours after PCI. Analyses were performed by intention to treat.

Results

At 1 year after STEMI, 485 patients had complete follow-up. The rate of the composite end point of all-cause mortality, re-infarction, target vessel revascularization, stroke and/or heart failure was 6.4% (N = 15) in the epoetin alfa group and 9.6% (N = 24) in the control group (p = 0.18). Thromboembolic events were present in 1.3% (N = 3) of patients in the epoetin alfa group and 2.4% (N = 6) in the control group. There was no evidence of benefit from epoetin alfa administration in subgroups of patients.

Conclusions

Administration of a single bolus of epoetin alfa in patients with STEMI does not result in a reduction of cardiovascular events at 1 year after primary PCI. There was a comparable incidence of thromboembolic complications in both treatment groups, suggesting that epoetin alfa administration is safe at long term.

Introduction

Preclinical studies have suggested that erythropoiesis- stimulating agents (ESA) have a cardioprotective effect after myocardial reperfusion. Subsequently, several clinical studies investigated the effects of ESA administered in patients with ST-elevation myocardial infarction (STEMI) undergoing primary percutaneous coronary intervention (PCI) to reduce myocardial infarct size and to improve left ventricular function.[1-9] However, the majority of these studies did not show a beneficial effect of ESA on left ventricular function. One of these studies even reported an increase in thromboembolic events in the ESA treated patients.[7] In addition, results suggested that ESA administration might have an adverse effect on infarct size among STEMI patients aged 70 years or older.[7] However, the number of STEMI patients in the clinical ESA trials was often small.

The HEBE III study was the largest, prospective study randomizing 529 STEMI patients to a single bolus of epoetin alfa or to standard medical care after primary PCI.[2] The primary endpoint of the HEBE III study was left ventricular function at 6 weeks after myocardial infarction. The single high dose of epoetin alfa failed to show an effect on the primary endpoint. However, patients receiving epoetin alfa had a lower 6-weeks incidence of adverse cardiovascular events. The aim of this follow-up study of HEBE III was to evaluate the effect of epoetin alfa on clinical outcome during the first year after myocardial infarction.

Methods

Population

The HEBE III study was a multicenter, randomized, open-label trial with blinded evaluation of the primary end point. The detailed study design and the first results of the primary end point at 6 weeks have been previously published.2,10 The aim of the study was to investigate the effect of high dose epoetin alfa administration after primary PCI on left ventricular function in patients with STEMI. Between January 2007 and June 2009, 529 patients were enrolled.

In short, patients were eligible for participation if they presented with a first STEMI with thrombolysis in myocardial infarction (TIMI) flow 0 or 1 on the coronary angiogram before the PCI procedure and underwent a successful primary PCI with TIMI flow 2 or 3 after PCI. STEMI was defined as chest pain suggestive of cardiac ischemia with symptom onset < 12 h before hospital admission or < 24 h in case of ongoing ischemia, an electrocardiogram with ST-T segment elevation > 0.1 mV in ≥ 2 or more leads or a new left bundle branch block. The most important exclusion criteria were a previous myocardial infarction, hemoglobin levels > 17.1 g/dL before PCI, anticipated additional

revascularization within 6 weeks after primary PCI, a history of persistent or permanent atrial fibrillation, cardiogenic shock and a serum creatinine > 2.5 mg/dL.

Randomization and treatment

After a successful primary PCI with TIMI flow 2 or 3 on the coronary angiogram after PCI, STEMI patients who met eligibility criteria were asked for participation. Patients were randomized (1:1) to optimal standard medical treatment with or without a bolus of 60.000 IU epoetin alfa (Ortho Biotech, a division of Janssen-Cilag B.V.) administered intravenously in 10 minutes. Patients received the bolus of epoetin alfa in the coronary care unit within 3 hours after PCI. Blood pressure, heart rate and ECG were constantly monitored, continuing at regular time points after the infusion up to 48 h after PCI.
All patients received aspirin (500 mg), heparin (5000 IU) and clopidogrel (600 mg) after confirmation of ST-segment elevation on the first ECG. During primary PCI, patients received the glycoprotein IIb/IIIa inhibitor abciximab (0.25 mg/kg) if not contraindicated. The standard treatment after primary PCI consisted of aspirin, clopidogrel (≥ 1 month), β blockers, lipid lowering agents, and angiotensin- converting enzyme inhibitors or angiotensin-II receptor blockers. The patients included in the study provided written informed consent. The research protocol was approved by the central Ethics Committee of the University Medical Center Groningen, and by the local Ethics Committees of each of the participating centers.

Outcomes and definitions

Information on vital status, re-infarction, target vessel revascularization and stroke was collected from hospital records and telephone interviews at 6 weeks and at 1 year after primary PCI. Re-infarction was defined as the onset of recurrent symptoms of ischemia combined with new ST-segment elevations and/or a second increase of serum CK or CK-MB to at least twice the upper limit of the normal range. Target vessel revascularization was defined as PCI or bypass grafting of the infarct-related coronary artery after primary PCI. Other end points included all-cause mortality, stroke and admission for heart failure. The composite end point of cardiovascular events was defined as all-cause mortality, re-infarction, target vessel revascularization, stroke and admission for heart failure. The composite end point of thromboembolic events was defined as re-infarction or stroke.

Statistical analysis

Data were analyzed on an intention to treat basis. Categorical variables are presented

as frequency values and proportions and were compared with the χ^2 test or Fisher's exact test. Continuous normally distributed variables are presented as mean values and standard deviations (SD) and were compared with the 2-tailed Student t test. For skewed distributed variables, median values with interquartile ranges are shown, and the variables were compared with the use of the Mann- Whitney U test. The cumulative incidence of the composite end point of all-cause mortality, re-infarction, target vessel revascularization, stroke and heart failure during the first year was evaluated with the Kaplan Meier method. The clinical outcomes in the treatment groups were compared with the log-rank test. Subgroup analyses were performed for gender, age > 70 years, diabetes, hypertension, smoking and time from symptom onset to PCI > 180 minutes, by means of logistic regression analysis, presenting risk ratios (RR) and corresponding 95% confidence intervals. For all analyses, 2-sided p values of <0.05 were defined as significant. Statistical analyses were performed using the Statistical Package of Social Sciences version 20.0 (SPSS, IBM corporation, Armonk, NY, USA).

Results

Study population

A total of 529 patients were randomized to epoetin alfa (N = 263) or to the control group (N = 266) (Figure 1). A total of 22 patients in the epoetin alfa group and 11 in the control group prematurely withdrew their informed consent, and we were therefore not allowed to report follow-up data of these patients. In 485 patients, 1 year follow-up was available, of which 236 patients were randomized to epoetin alfa and 249 patients

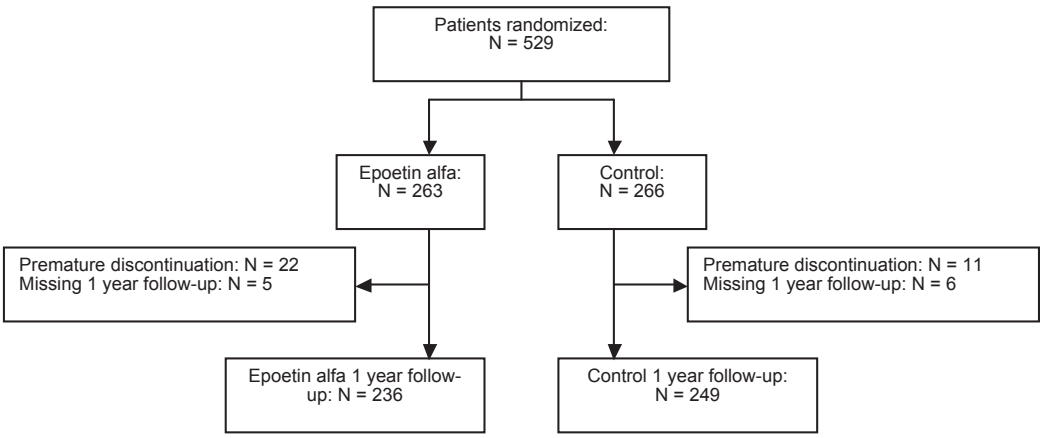

Figure 1. Flow chart. Flow chart of STEMI patients randomized to epoetin alfa or the control group

Table 1. Baseline clinical and angiographic characteristics

	Epoetin alfa N = 236	Control N = 249
Age (years) (± SD)	60.1 (± 10.5)	60.5 (± 11.0)
Male gender	180 (76.3%)	199 (79.9%)
Diabetes	25 (10.6%)	18 (7.2%)
Hypertension	78 (33.2%)	83 (33.3%)
Hypercholesterolemia	51 (21.7%)	43 (17.3%)
Family history CAD	120 (51.1%)	131 (52.6%)
Current smoker	56 (24.0%)	56 (22.5%)
Prior revascularization	5 (2.1%)	8 (3.2%)
Systolic blood pressure (mmHg) (± SD)	127.9 (± 24.8)	129.6 (± 23.6)
Diastolic blood pressure (mmHg) (± SD)	76.7 (± 14.8)	77.1 (± 13.9)
Heart rate (b.p.m.) (± SD)	75.2 (± 15.6)	74.0 (± 16.1)
Haemoglobin at baseline (g/dL) (± SD)	14.0 (± 1.4)	14.3 (± 1.3)
Haematocrit at baseline (%) (± SD)	40.2 (± 3.8)	41.3 (± 3.5)
Serum creatinine at baseline (mg/dL) (IQR)	0.85 (0.74-0.98)	0.87 (0.76-1.00)
Time from symptom onset to PCI (min) (IQR)	180 (126-290)	175 (120-255)
Number of diseased vessels		
1	157 (66.8%)	168 (67.5%)
2	60 (25.5%)	62 (24.9%)
3	18 (7.7%)	19 (7.6%)
Infarct-related coronary artery		
LAD	96 (40.9%)	99 (39.8%)
Cx	38 (16.2%)	42 (16.9%)
RCA	101 (43.0%)	107 (43.0%)

b.p.m = beats per minute, CAD = coronary artery disease, Cx = circumflex coronary artery, IQR = interquartile range, LAD = left artery descending, PCI = percutaneous coronary intervention, RCA = right coronary artery

to the control group. The baseline clinical characteristics were well balanced between the treatment groups (Table 1). The mean age of the patients was 60.1 (±10.5) years in the epoetin alfa group and 60.5 (±11.0) years in the control group, and the proportion of males was 76.3% versus 79.9%. The median time from symptom onset to PCI was 180 (interquartile range 126- 290) minutes in patients receiving epoetin alfa and 175 (interquartile range 120- 255) minutes in patients in the control group. The hemoglobin level at baseline was 14.0 (±1.4) g/dL in the epoetin alfa group and 14.3 (±1.3) g/dL in the control group. The majority of patients presented with 1 vessel disease (66.8% versus 67.5%) on the coronary angiogram.

Clinical outcomes

A total of 6 patients died during 1 year follow-up after primary PCI, 1 patient in the epoetin alfa group and 5 in the control group (Table 2). The composite end point of all-cause mortality, re-infarction, target vessel revascularization, stroke and heart failure occurred in 6.4% (N = 15) of patients in the epoetin alfa group and 9.6% (N = 24) of

Table 2. Clinical events at 1 year follow-up

	Epoetin alfa N = 236	Control N = 249	p value
All -cause mortality	1 (0.4%)	5 (2.0%)	0.12
Re- infarction	1 (0.4%)	5 (2.0%)	0.12
Target vessel revascularization	10 (4.2%)	13 (5.2%)	0.61
Stroke	2 (0.8%)	1 (0.4%)	0.53
Heart failure	1 (0.7%)	4 (2.6%)	0.19
Composite end point	15 (6.4%)	24 (9.6%)	0.18
Thromboembolic complications	3 (1.3%)	6 (2.4%)	0.35
Major bleeding	8 (3.4%)	10 (4.0%)	0.72

The composite end point includes all-cause mortality, re-infarction, target vessel revascularization, stroke and heart failure. Thromboembolic complications include re-infarction and stroke

patients in the control group (p = 0.18) during the first year after primary PCI (Table 2, Figure 2). Thromboembolic events were present in 1.3% (N = 3) of patients in the epoetin alfa group and 2.4% (N = 6) in the control group. Major bleeding occurred in 3.4% (N = 8) and 4.0% (N = 10) of patients, respectively.

Subgroups

In the subgroups of gender, age (>70 years), diabetes, hypertension, smoking and time from symptom onset to primary PCI (>180 minutes), there was no significant difference in the incidence of the composite end point between the epoetin alfa group and the control group (Figure 3).

Discussion

The 1 year follow-up results of this randomized trial on epoetin alfa in 485 STEMI patients showed no significant difference in the composite end point of all-cause mortality, re-infarction, target vessel revascularization, stroke and heart failure between STEMI patients randomized to a single high dose epoetin alfa or to standard optimal medical treatment after primary PCI. In addition, the incidence of thromboembolic complications was comparable between the treatment groups.

Erythropoietin is a hematopoietic hormone produced by the kidneys in response to hypoxia. It stimulates the production of red blood cells by inhibition of apoptosis of progenitor cells, causing an increase of the hemoglobin level. In clinical practice, exogenous erythropoietin is often administered in anemic patients with a reduced erythropoietin production.[11]ESA are mainly used in anemic patients with chronic kidney disease and cancer, and in these patients chronic administration effectively raises hemoglobin leading to a reduction in symptoms. However, this is not associated with a favorable ef-

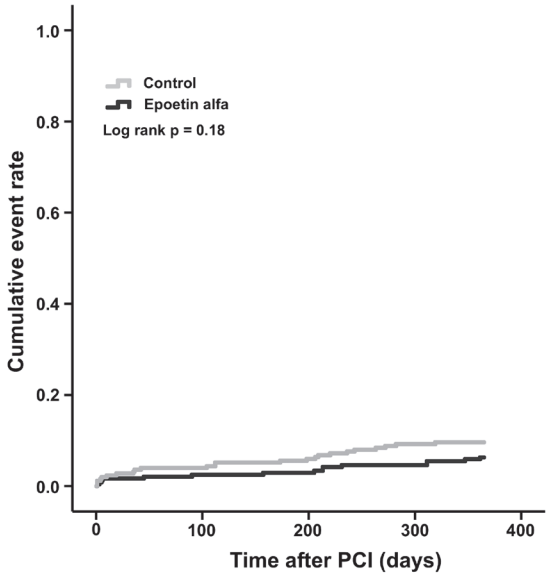

Figure 2. Cumulative incidence of the composite end point all-cause mortality, re-infarction, target vessel revascularization, stroke and heart failure. Cumulative event rate of STEMI patients randomized to epoetin alfa or the control group during the first year after primary PCI

fect on outcome.[12-14] Long-term use of ESA have also been investigated in patients with chronic heart failure. In these patients, it reduced symptoms and increased exercise capacity,[15,16] but it also did not improve outcome.[17]

ESA have also been investigated in acute ischemic conditions in patients without anemia. In these patients, single doses of ESA were used with the aim of protection against ischemia. In patients with acute cerebral ischemia early studies suggested potentially beneficial effects of ESA.[18] However, a subsequent much larger study indicated that ESA were not beneficial in these patients.[19] In comparison, more studies have been conducted in patients with acute cardiac ischemia and myocardial infarction as compared to acute cerebral ischemia. In preclinical studies, administration of ESA showed cardioprotective effects in cardiac ischemia.[20] Administration of exogenous erythropoietin resulted in a reduction of cardiac cell apoptosis in different animal models in rats and rabbits during ischemia, and after the onset of reperfusion.[21-23] Administration of ESA even resulted in a reduction in cardiomyocyte loss and a decreased infarct size after induced ischemia in rats and dogs.[21,24,25] In addition, ESA may contribute to a reduction in the inflammatory response induced after myocardial reperfusion and have a positive effect on neovascularization.[25,26]

After these encouraging pre-clinical studies, clinical trials have evaluated the effect of ESA administration in patients with STEMI undergoing PCI.[1-9] In 2 small clinical stud-

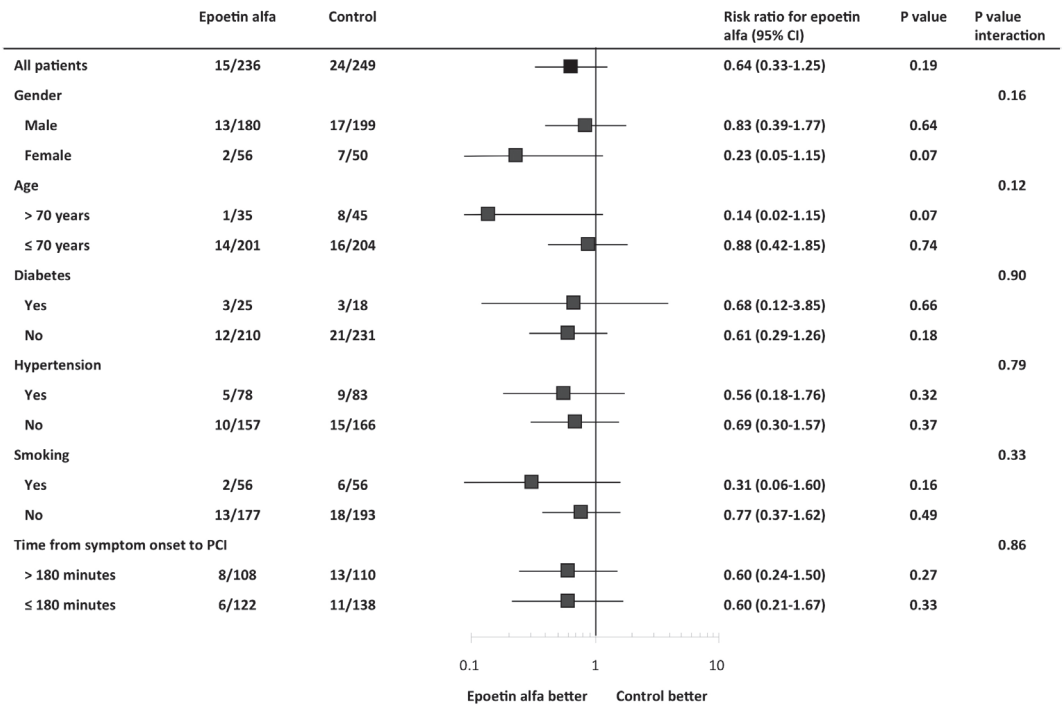

Figure 3. Subgroup analysis Subgroup analysis of the composite end point of all-cause mortality, re-infarction, target vessel revascularization, stroke and heart failure

ies, the investigators observed an improvement in left ventricular function over time in STEMI patients receiving ESA, and not in the control group.[4,5] Although the results of preclinical studies were promising, ESA administration did not result in a reduction of infarct size or an improvement in left ventricular function between the treatment groups in most clinical trials. We performed the largest randomized clinical trial investigating the effect of epoetin alfa in STEMI patients so far. Epoetin alfa did not result in an improvement of left ventricular function at 6 weeks after primary PCI. However, we observed a reduction in cardiovascular events at 6 weeks in the epoetin alfa group compared to the control group, although numbers were low (8 vs 19, p = 0.032). In the current study we can report that one year after PCI, the initial differences in cardiovascular events disappeared.

Regarding the overwhelming effects in animal models of acute myocardial infarction, how can the discrepancy with the clinical situation be explained?[27] A first explanation may be that the cardioprotective effect of ESA in animals cannot be translated to humans, probably as a consequence of differences in coronary anatomy, and differences in infarct sizes and ESA dose compared with clinical studies. Secondly, the timing of epo-

etin alfa administration may not have been optimal, as epoetin alfa was administered after myocardial reperfusion.[28] The higher ESA doses used in animals compared to humans could have contributed to the observed differences in treatment effects. However, it seems that the dose of epoetin alfa was high enough to have effects on serum levels, as a previous pilot study showed a 200-fold increase of serum erythropoietin levels after administration of high dose darbepoetin alfa.[29] Third, a single bolus dose of epoetin alfa may not have been sufficient. However, it should be taken into account that increases in hemoglobin levels may cause unwanted side effects.[30] Fourth, we included patients with a first STEMI who were reperfused early after symptom onset. As a consequence of the small infarct sizes in a majority of patients, the possible effects of epoetin alfa may not have been observed. Finally, the number of included patients may have been too small to observe an effect on clinical outcome.

Safety

Administration of high dose ESA has raised safety concerns in patients with anemia and chronic kidney disease receiving high ESA doses. In a study of patients with diabetes, chronic kidney disease and anemia, high ESA doses resulted in an increased risk of stroke in patients receiving darbepoetin alfa compared to placebo.[14] In addition, some randomized trials in STEMI patients observed a trend towards a higher incidence of re-infarction, target vessel revascularization and stroke in patients treated with ESA compared to placebo, but numbers were low.1,7 In the present larger follow-up study of HEBE III, the incidence of cardiovascular events did not significantly differ between patients randomized to epoetin alfa or the control group at 1 year after primary PCI. This is also supported by the results of a meta-analysis of randomized trials investigating the effect and safety of ESA in patients with myocardial infarction.[31] However, this was performed on study level rather than with individual patient data.

Subgroups

Najjar et al. observed that in patients ≥ 70 years the mean infarct size was larger in the ESA group than in the placebo group on the first cardiac magnetic resonance imaging.7 We did not observe a higher incidence of adverse cardiovascular events in patients ≥ 70 years receiving ESA compared to the control group. In addition, none of the investigated subgroups showed a significant difference in cardiovascular events between the treatment allocations.

Limitations

Several limitations should be taken into account. This study was powered on an estimated improvement of left ventricular ejection fraction at 6 weeks after PCI. Therefore, the number of patients may have been too small to investigate a difference in clinical outcome and cardiovascular events at 1 year after PCI. In addition, the number of events was small, making it difficult to observe differences in effect between the treatment allocations. Furthermore, the population of STEMI patients included in this study was a selective population with better clinical outcomes than expected in a general STEMI population. This makes it more difficult to translate our results to real world clinical practice. Finally, the study was not blinded for treatment allocation.

Conclusion and implications

The administration of epoetin alfa in patients with STEMI did not result in a reduction of cardiovascular events at 1 year after primary PCI. However, the higher incidence of thromboembolic complications, as observed in some previous studies, was not observed in patients receiving epoetin alfa in this study. This may suggest that a single dose of epoetin alfa administration is safe at the long term. However, whether serial ESA administration in STEMI patients is safe and effective should be investigated in an adequately powered trial with clinical end points.

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Chapter 8

Erythropoeitin and heart failure: the end of a promise?

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For over a decade, anemia has been explored in heart failure (HF) patients. Anemia is observed in 25-40 % of HF patients depending on severity of disease and definition of anemia. Furthermore, its presence is associated with increased mortality and HF hospitalizations.[1] In patients with anemia, mortality risk is approximately doubled. [2] The etiology of anemia in heart failure is multifactorial, including inflammation, medication, malnutrition, iron deficiency, renal insufficiency, hemodilution, and erythropoietin (EPO) resistance.[3-7]

Anemia is one of the reversible comorbidities associated with HF and correction is possible with erythropoiesis stimulating agents (ESA). Small studies indicated that treatment with ESA might reduce heart failure hospitalizations and improve exercise capacity and quality of life through correction of anemia.[8-10] In a meta-analysis, patients treated with ESA showed a decreased risk of hospitalization for HF.[11] To provide a final answer to the question whether ESA therapy in patients with anemia and heart failure is appropriate, the Reduction of Events With Darbepoetin Alfa in Heart Failure Trial (RED-HF) was designed.[12]

Inclusion criteria of the RED-HF were HF of at least 3 months duration and of NYHA class II or more, hemoglobin levels between 9.0 g/dL and 12.0 g/dL and a left ventricular ejection fraction equal to or less than 40%. Patients with iron deficiency, defined as a transferrin saturation less than 15% were excluded. Furthermore, hypertension (blood pressure over 160/100 mm Hg) and renal dysfunction (serum creatinine > 265 μmol/L) or dialysis were major exclusion criteria.

The RED HF trial started including in 2006 and its primary endpoint was to determine the efficacy of darbepoetin alfa compared to placebo on the composite endpoint of time to death from any cause or first hospital admission for worsening HF in subjects with symptomatic left ventricular systolic dysfunction and anemia. Secondary endpoints included the effect of darbepoetin treatment on time to death from any cause, time to cardiovascular death or first admission for worsening HF and change in quality of life from baseline to 6 months measured by Kansas City Cardiomyopathy Questionnaire (KCCQ).

After 6 years of inclusion, 2278 patients were enrolled in 453 sites. Baseline characteristics did not differ between treatment and placebo groups.[13] Patients had severe HF; about two thirds were in NYHA class III or IV. In the treatment group, median hemoglobin levels increased from 11.2 g/dL to 13 g/dL. Nevertheless, no difference in the primary composite endpoint of death or heart failure hospitalizations between treatment and placebo arms could be demonstrated.(HR 1.01, 95% confidence interval, 0.90 to 1.13; P=0.87).[14] Fatal or nonfatal stroke occurred in 42 patients (3.7%) in the darbepoetin

alfa group and 31 patients (2.7%) in the placebo group (P=0.23). Furthermore, all cause thromboembolic events were reported in 153 patients (13.5%) in the darbepoetin alfa group and 114 patients (10.0%) in the placebo group (P=0.01). Darbepoetin alfa treatment was associated with a significant improvement in quality of life, as evidenced by a 2.2 point higher KCCQ score compared to the placebo group (P=0.005). However, the clinical significance of such a moderate improvement remains unclear.

The Trial to Reduce Cardiovascular Events With Aranesp® Therapy (TREAT) was the first study to evaluate the effect of chronic ESA treatment on cardiovascular events, although in patients with chronic kidney disease.[15] The results showed that especially when patients had a poor initial hemoglobin response, ESA was associated with an increased incidence of the composite cardiovascular endpoint of death, myocardial infarction, stroke, heart failure or hospitalization for myocardial ischemia (HR 1.31; 95% CI 1.09-1.59).[16] ESA treatment was also associated with an increased risk of stroke (HR 1.26; 95% CI 0.78–2.02). This finding is of relevance since in a previous study in which ESA was given to patients after a stroke, active treatment was associated with an increased incidence of stroke recurrence.[17] The RED-HF trial corroborates with these results as thromboembolic adverse events occurred significantly more in the active treatment group than in the placebo group. Although not reaching statistical significance, more fatal and non fatal strokes were also observed in the treatment group.(fig 1.)

The mechanism through which ESA is associated with adverse events is poorly understood. Several mechanisms can be hypothesized. First, ESA increases hematocrit levels by stimulating erythroid precursor production in the bone marrow and could therefore influence the rheology of the blood. Second, ESA are known for its pleiotropic effects including neovascularisation but these pleiotropic effects could also promote untoward effects including endothelial dysfunction, or hypercoagulability as well.

Despite the equivocal effects on the primary endpoint of death or HF hospitalizations and despite the slight increase in thromboemolic events, it remains unknown whether there are subgroups of HF patients that may benefit from ESA treatment. Earlier studies showed that patients with inappropriate high endogenous EPO levels and anemia are at the highest risk, probably reflecting bone marrow depression as a result of end stage disease or chronic inflammation.[18,19] Further subgroup analysis may reveal that patients requiring only a low dose EPO to restore their hemoglobin values may have benefitted from ESA treatment, as their bone marrow is more responsive. More support for low dose EPO comes from research exploring extrahematopoeitic effects; regardless of hematopoietic respons, ESA are able to induce neovascularization.[20] As capillary densitiy in HF patients is decreased, these patients may still benefit from low dose ESA. The first

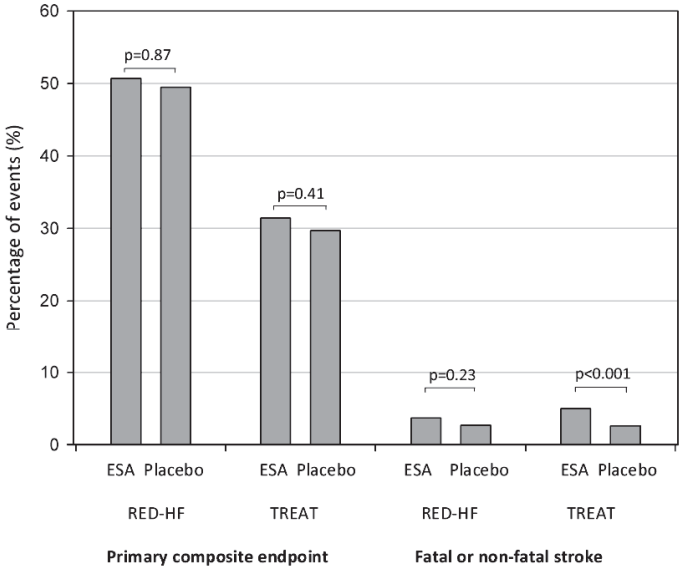

Fig 1. Percentage of primary composite endpoint and fatal and non fatal stroke in RED-HF and TREAT.

clinical trials in patients with myocardial infarction remain however neutral.

Another important anemia associated target is iron deficiency.[21] The earlier published FAIR-HF trial showed that correction with ferric carboxymaltose in patients with HF, even in the absence of anemia, improves functional capacity and quality of life.[22] As ESA treatment in HF does not improve outcome, one might therefore speculate that anemia in heart failure is a marker of severity of disease, rather than a mediator. On the other hand, ESA could elicit negative effects on its own, thereby offsetting possible beneficial effects of increasing hemoglobin levels.

In conclusion, RED-HF shows that increasing hemoglobin concentrations with ESA does not reduce morbidity and mortality in anemic HF patients. Anemia may therefore be a marker of disease severity rather than a therapeutic target in patients with HF and ESA treatment is therefore not recommended.

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Chapter 9

Summary, conclusions and future directions

Anemia is an established risk factor for mortality and morbidity in patients with cardiovascular disease. As the impact of cardiovascular disease on hospitalization, mortality and economic burden is enormous, new treatment regimens are warranted. Understanding the pathophysiological processes involved in the anemia of cardiovascular disease may reveal novel therapeutic options. One of the therapeutics currently under investigation is erythropoietin (EPO), a hematopoietic hormone that has extra-hematopoietic effects as well. In **chapter 1** we discuss the current knowledge concerning the etiology of anemia and the role of EPO in cardiovascular disease and provide a rationale for the aims of the current thesis. The current thesis aims to explore the etiology of anemia in cardiovascular disease and to evaluate EPO treatment.

Anemia and cardiovascular disease

Several factors play a role in the etiology of anemia in patients with cardiovascular disease. In the first part of this thesis we investigated the role of congestion, inflammation and angiotensin converting enzyme (ACE) inhibitors in patients with cardiovascular disease. First, we aimed to assess central venous pressure in a broad spectrum of cardiovascular patients.(**chapter 2**) In 2009 consecutive patients who underwent right heart catheterization laboratory data were extracted and anemic patients were identified. Approximately 27.4% of the patients were anemic and these anemic patients had higher central venous pressure, higher cardiac index and lower systemic vascular resistance. Furthermore, we showed that anemia and increased central venous pressure were associated with an over two fold higher mortality rate. We concluded that anemia is common in cardiovascular patients and independently related to an elevated central venous pressure and cardiac index. Furthermore, patients with both elevated central venous pressure and anemia have the worst prognosis, independent of cardiac index.
In **chapter 3** we evaluated the role of inflammation in the presence of anemia in chronic heart failure patients. In 325 patients, levels of inflammatory factors interleukin-6, soluble tumor necrosis factor receptor 1 and high sensitive C-reactive protein and hemoglobin were assessed. In this heart failure cohort, 40% of the patients were anemic. Anemic patients had significant higher levels of interleukin-6, high sensitive C-reactive protein and soluble Tumor Necrosis Factor Receptor 1. In multivariable analysis, higher levels of high sensitive C-reactive protein and soluble Tumor Necrosis Factor Receptor 1 were significantly associated with anemia. Cox regression analysis revealed soluble Tumor Necrosis Factor Receptor 1 as a significant predictor of mortality. From these data we may conclude that these inflammatory cytokines, could be responsible for the development of anemia in heart failure patients and play a significant role in the mortality of

heart failure patients in the presence of anemia.

Despite all efforts to prevent post-operative anemia, more than 90% of patients develop anemia after coronary artery bypass grafing (CABG) surgery. [1] These cardiovascular patients are thus subjected to an established cardiovascular risk factor by an intervention that is intended to reduce that risk. In **chapter 4** we evaluated whether the severity and the degree of postoperative anemia affects prognosis in a cohort of 2553 stable patients with left ventricular ejection fraction >40%, two to seven days after scheduled CABG who were randomized between the ACE inhibitor Acupril or placebo. In 43% of the patients, anemia persisted for more than 50 days. These patients with sustained post-operative anemia displayed a markedly impaired prognosis. In fact, every mg/L decrease in hemoglobin was associated with an 11% increase in the primary composite endpoint of death, myocardial infarction, stroke, heart failure and angina. Furthermore, patients randomized to Acupril had slower recovery of hemoglobin levels. We concluded that postoperative anemia is common, frequently persists for months after CABG surgery and is associated with an impaired outcome. In patients with anemia, ACE inhibitors slowed recovery from postoperative anemia and increased the incidence of cardiovascular events after CABG surgery.

To further explore whether bone marrow dysfunction could be responsible for prolonged postoperative anemia we analyzed reticulocyte count and biochemical markers in a cohort of 1147 patients who underwent scheduled CABG surgery.(**chapter 5**) The increase in reticulocyte count after CABG surgery was inversely related to changes hemoglobin and C-reactive protein. In 11% of the patients, reticulocyte count did not increase following the anemic state of surgery. We observed that that impaired erythropoiesis after CABG surgery is associated with an increased inflammatory response. This could be a clue in the etiology of sustained impaired erythropoiesis response following thoracic surgery.

Erythropoietin in cardiovascular disease

Several studies have evaluated the treatment of EPO in cardiovascular patients. First, in experimental setting EPO not only showed to have hematopoietic effects but cardioprotective pleiotropic effects as well in animals subjected to myocardial infarction. Both anti-apoptotic properties and neovascularisation owe to these effects.[2,3] In clinical trials however, EPO therapy does not results in beneficial cardiac remodeling.[4] One of these studies performed to evaluate EPO to preserve cardiac function was the HEBE III study.[5] As EPO therapy could actually result in thromboembolic events due to increased blood viscosity, we performed a one year follow up to evaluate readmissions and thrombo-

embolic events in the HEBEIII study.(**chapter 7**) In total, of the 529 patients, 485 patients had complete follow up. The composite end point of all-cause mortality, re-infarction, target vessel revascularization, stroke and/or heart failure was comparable between the treatment groups. Furthermore, there was a comparable incidence of thromboembolic complications in both treatment groups, suggesting that EPO treatment is safe at long term.

A further concern to treatment with EPO was raised in patients with heart failure. Depending on the definition used, up to 40% of these patients are anemic. As the presence of anemia in these patients is associated with morbidity, mortality and functional status, correcting anemia to improve outcomes seems plausible.[6] In contrast to a single bolus of EPO in studies evaluating this drug in patients with myocardial infarction, frequent administration is needed in patients with anemia in heart failure. This raised the question whether rheologic effects of EPO could actually induce harm as higher blood viscosity could lead to thromboembolic events. In the **chapter 6** we give comment on these possible effects and the therapeutic window of erythropoietin. In 2013, the long awaited RED-HF trial was published. In this randomized, double-blind trial, 2278 patients with systolic heart failure and mild-to-moderate anemia (hemoglboin level, 9.0 to 12.0 g per deciliter) were randomized to receive either darbepoetin alfa (to achieve a hemoglobin target of 13 g per deciliter) or placebo. The primary outcome was a composite of death from any cause or hospitalization for worsening heart failure. Small studies had already shown a beneficial effect, although trials with EPO in non-cardiac studies showed an increase in thromboembolic events.[7] No differences could be observed in the primary endpoint. Patients treated with darbepoietin did however have an improvement in quality of life. In **chapter 8** we comment on the RED-HF study.

Future directions

In the last decade, anemia has been increasingly recognized as a therapeutic target in cardiovascular patients to improve survival and quality of life. Neutral results of the large phase 3 trial RED-HF indicate however, that other avenues should be explored.[8] Although multiple mechanisms attribute to anemia in cardiovascular patients, work from this thesis indicates that inflammation and fluid retention are important and prognostic relevant etiologies for anemia in the cardiovascular patient, suggesting that therapeutic targets focusing on these entities might prove beneficial.

Inflammation

In the nineties, tumor necrosis factor alpha (TNF-α) was discovered as an important biomarker in patients with heart failure.[9] As it seemed to be involved in unfavorable remodeling, clinical trials with a TNF-α inhibitor (infliximab) in patients with heart failure followed subsequent. Despite the therapeutic promise, infliximab adversely affected patients with moderate to severe heart failure, despite effectively lowering TNF-α and interleukin [6.10,11] Recent studies also showed that patients with myocardial infarction who were administered infliximab did not have clinical benefit.[12] These trials unfortunately did not assess hemoglobin or anemia. Other trials investigating infliximab in patients with other chronic disease, rheumatoid arthritis and inflammatory bowel disease, did however reveal an improvement in hemoglobin levels, suggesting that TNF alpha inhibition could be an effective treatment for the anemia of chronic disease.[13,14] The paradoxal results of the earlier studies with infliximab in patients with heart failure could be explained by a recent discovery. Experimental data reveals that two isoforms of the tumor necrosis factor receptor posses different effects. Myocardial injury is mediated by the tumor necrosis factor receptor type 1, whereas beneficial effects are mediated trough the tumor necrosis factor receptor type 2.[15] Infliximab, which does not selectively inhibit one of these receptors, thus has ambivalent effects. Future studies should be aimed to explore the mechanism of the tumor necrosis factor receptor type 1, and the effects of its selective inhibition on anemia and cardiovascular disease.

Congestion

Fluid congestion is a hallmark symptom of the heart failure syndrome. Increasing venous pressure causes fluid to transude out of capillaries into tissue spaces faster than lymphatics can drain the fluid away, eventually causing edema. In chronic heart failure, decreased renal perfusion causes activation of the renin-angiotensin alosterone system (RAAS), resulting in fluid retention and increase in extracellular volume. Furthermore, increased levels of antidiuretic hormone may lead to fluid retention. Eventually, this fluid overload causes hemodilution, resulting in a state of pseudoanemia.[16,17] Despite the relation between anemia and fluid retention, signs and symptoms were absent in these studies. It thus seems that hemodilution precedes clinical presentation of fluid overload. In this thesis we additionally show that when anemia is present on top of increased venous pressure, patients have a twofold risk of mortality, further underlining that anemia is marker of severity of disease. Studies investigating and targeting fluid retention are therefore warranted. A study with direct measurement of blood volume and additionally guided therapy using novel radiolabeled dye dilution techniques is

currently tested in the ongoing TEAM-HF (Treating to Euvolemia by Clinical Assessment and Measured Blood Volume in Heart Failure) trial. [18]

Another important anemia related therapeutic target is iron deficiency. Even independent of the presence of anemia, iron deficiency is related to impaired quality of life and prognosis.[19] Iron is not only an important integral component of hemoglobin, it is important in oxygen storage in myoglobin and cellular energy production in muscles.[20,21] Therefore, iron suppletion is proposed as an important therapeutic target independent of anemia or as an alternative to eythropoiesis stimulating agents such as EPO.[19] Indeed, in the FAIR-HF trial, iron repletion resulted in improvement of functional capacity and quality of life.[22]. With neutral effects of the RED-HF, one could therefore speculate whether anemia is only a marker of vulnerable heart failure patients. Furthermore, iron metabolism could be a major link between anemia and inflammation as proinflammatory cytokines are involved in the synthesis of hepcidin, a hormone that in turn regulates intestinal iron absorption and tissue distribution by inducing degradation of the cellular iron exporter ferroportin.[23,24] Future research could thus focus on these mediators. Several trials are currently investigating iron suppletion on outcome in heart failure patients.

In the current thesis we provide evidence to support the notion that sustained postoperative anemia should not be considered as a benign disease as mortality rates are doubled. Therefore, standard diagnostic evaluation and treatment of anemia before discharge might improve outcome after CABG. In addition, increased utilization of contemporary strategies to prevent allogenic blood transfusions, such as minimal invasive surgery, autologous blood transfusions, thrombostatic drugs and erythropoiesis stimulating proteins might limit postoperative anemia. Alternatively, sustained postoperative anemia might represent a marker for a high risk population. Second, patients scheduled for CABG surgery with active chronic inflammation could represent an even higher risk group and especially these patients should be intensively monitored to limit the duration of post-operative anemia after CABG surgery. In addition, it would be prudent to avoid erythropoiesis inhibiting factors such as angiotensin convering enzyme (ACE) inhibitors in the early post operative phase.[25]

Finally, it seems that anemia is a marker of disease rather than a mediator and therefore identifies the vulnerable cardiovascular patient. First, anemia should be recognized as such and proper evaluation including hematinic deficiencies and reticulocytes should be performed in order to improve morbidity.[26] Second, new studies are needed to investigate mechanistic causes of anemia in cardiovascular patients to improve morbidity and mortality.

Erythropoeitin and cardiovascular disease

Recombinant EPO has been used for over three decades in patients with chronic kidney disease, resulting in improved quality of life and decrease in blood transfusions.[27] It was increasingly recognized as a pleiotropic cytokine in 2002. In experimental setting EPO improved infarct size and left ventricular function following myocardial infarction.[28,29] The first clinical studies with EPO treatment in heart failure were promising and treatment was safe.[30,31] EPO treatment showed a trend towards reduction in mortality and first hospitalizations for heart failure. These observations were contradictory to other patients groups in which EPO treatment was investigated. In patients with acute ischemic stroke for example, EPO was associated with a higher death rate.[32] Furthermore, in the TREAT (Trial to Reduce Cardiovascular Events with Aranesp Therapy) study, patients with chronic kidney disease had increased risk of stroke.[7] In a subanalysis of the CHOIR (Correction of Hemoglobin and Outcomes in Renal Insufficiency) study, also performed in patients with chronic kidney disease, uptitration of EPO to higher levels of hemoglobin resulted in increased risk of death, myocardial infarction, congestive heart failure or stroke. In the RED-HF study, chronic treatment with erythropoietin did result in more frequent thromboembolic events, although stroke did not occur more often in the EPO group.[33] In myocardial infarction, however, a single bolus of EPO did not result in more major adverse events.[5] In addition, in this thesis we show that up to 1 year after administration, a single bolus of EPO is safe. From these data we can speculate that timing and dosage of EPO is crucial with regard to effects and adverse effects. However, trials using low dose EPO are currently not available, it could thus be that EPO is still effective, but should be administered in lower doses. On the other hand, new erythropoietin derivates are being developed that do not possess hematopoietic effects.[34] These non-erythropoietic derivates retain the tissue protective properties without undesirable effects of erythropoiesis. Especially in chronic use these agents would be desirable. A recent experiment study showed that treatment with a small peptide sequence within the EPO molecule, helix B surface peptide, shows cardioprotection.[35] However, this agent is only studied by one study group, and clinical studies are lacking so far.

Results from the experimental EPO studies do not translate to the human setting. Several factors could be involved in these discordant results. For instance coronary anatomy of rodents is different than in humans, possibly also resulting in far larger infarct sizes in the experimental studies. Second, experimental studies do not take co morbidities and medication use into account. Dosage of erythropoietin is also different then in experimental setting, as higher cumulative doses of EPO were used. Finally, whereas EPO

could be administered directly following myocardial infarction in experimental setting, in human setting EPO could only be administered after a median time of four hours after onset of symptoms in the case of myocardial infarction. These factors should make us be cautions with interpreting experimental work into clinical setting.

Putting the knowledge of the current thesis and recent literature in perspective, it seems that anemia is merely a marker of severity of disease and that EPO, although effective in experimental setting does not translate into clinical application to either prevent myocardial damage in patients with myocardial infarction or improve prognosis in patients with heart failure.

In summary, the present thesis aimed to investigate associated factors for the development of anemia in cardiovascular disease and evaluate the therapeutic potential of EPO. Results from our study showed that fluid retention, inflammatory factors and bone marrow dysfunction could play an important role in the development of anemia in patients with heart failure, or following CABG surgery. Furthermore, our results show that a single bolus of EPO following myocardial infarction is safe. Future studies evaluating inflammation, volume status and other mediators of anemia in cardiovascular disease are warranted. Therapy of EPO in patients with heart failure or following myocardial infarction seems past, although knowledge of the mechanism of cardioprotection will lead to novel agents for the future.

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Appendices

Nederlandse samenvatiting

Anemie, beschrijft de ziekte waarbij er een tekort is aan circulerende rode bloedcellen. Het wordt vaak bepaald aan de hand van de hoogte van het Hemoglobine, een belangrijk bestandsdeel van de rode bloedcel. Anemie is een belangrijke risicofactor voor mortaliteit en morbiditeit bij patiënten met hart- en vaatziekten. Aangezien de gevolgen van hart- en vaatziekten voor ziekenhuisopnames, sterfte en kosten in de gezondheidszorg van groot belang zijn, zijn nieuwe behandelingen wenselijk. Het begrijpen van het ontstaan van anemie kan tot nieuwe therapieën leiden. Een van de therapieën die momenteel wordt onderzocht is erythropoëtine (EPO), een hormoon dat onder andere voor bloedaanmaak zorgt, maar ook andere gunstige effecten heeft. In **hoofdstuk 1** bespreken we de huidige kennis van anemie en therapie bij patiënten met hart- en vaatziekten. Tevens geven we de doelen weer van dit proefschrift. Dit proefschrift is erop gericht mogelijke oorzaken van anemie bij patiënten met hart- en vaatziekten te ontrafelen (deel 1) en om therapie met EPO te beoordelen (deel 2).

In het eerste deel van dit proefschrift bespreken we mogelijke oorzaken van anemie bij patiënten met hart- en vaatziekten. Ten eerste vonden we dat een verhoogde centraal veneuze druk een onafhankelijke voorspeller was voor anemie bij patiënten met hart- en vaatziekten van verschillende oorzaak. Bovendien hadden anemische patiënten met een verhoogde centraal veneuze druk een slechtere prognose dan patiënten waarbij de veneuze druk niet verhoogd was. (**hoofdstuk 2**). Ten tweede onderzochten we de samenhang tussen anemie en inflammatoire cytokines bij patiënten met hartfalen. We vonden dat hoog sensitieve C-reactief proteïne (hsCRP) waarden en soluble Tumor Necrosis Factor Receptor 1 (sTNFR-1) onafhankelijk geassocieerd zijn met het optreden van anemie (hoofdstuk 3). Ook was sTNFR-1 een onafhankelijke voorspeller voor sterfte bij deze patiënten met hartfalen. Ten derde onderzochten we het herstel van anemie postoperatief bij patiënten die een coronaire bypass operatie (CABG) hadden ondergaan. We toonden aan dat anemie vaak maanden na deze operatie blijft bestaan en dat bij patiënten die een angiotensine converting enzyme (ACE) remmer gebruiken het herstel van hemoglobinewaarden trager verliep. Bovendien hadden deze patiënten met een slechter herstel van anemie een verslechterde prognose. (**hoofdstuk 4**) Tot slot onderzochten we de beenmergfunctie bij patiënten die een coronaire bypass operatie hadden ondergaan, door te kijken naar de reticulocyten, voorlopercellen van erythrocyten, in het bloed. We laten zien dat een verminderde beenmergrespons en verhoogde ontstekingswaarden beide gepaard gaan met anemie. (**hoofdstuk 5**). Het kan dus dat ontsteking zorgt voor verminderde beenmergfunctie en daarmee

verminderd herstel van postoperatieve anemie. Concluderend laten we in dit deel van het proefschrift zien dat anemie bij patiënten met hart- en vaatziekten geassocieerd is met verminderde beenmergrespons, verhoogde centraal veneuze druk, verhoogde ontstekingswaarden en het gebruik van ACE-remmers.

Het tweede deel van dit proefschrift richt zich op de behandeling van patiënten met hart- en vaatziekten met EPO. Dit hormoon heeft twee bijzondere eigenschappen die onderzocht zijn bij patiënten met een acuut myocardinfarct. Enerzijds zorgt het voor vaatnieuwvorming, anderzijds kan het zorgen voor minder celverval. Grote studies lieten echter geen voordeel zien en er werd geopperd dat EPO door een verhoogde stollingsneiging juist schadelijk zou kunnen zijn. In **hoofdstuk 7** doen we verslag van een één jaar follow-up van een studie die EPO versus placebo bij een acuut myocardinfarct onderzocht. De samengestelde uitkomst van mortaliteit door alle oorzaken, re-infarct, revascularisatie, beroerte en/of hartfalen was vergelijkbaar tussen beide behandelgroepen. Verder was er een vergelijkbare incidentie van trombo-embolische complicaties in beide behandelingsgroepen, wat suggereert dat eenmalige EPO behandeling op lange termijn veilig is.

Aangezien bij patiënten met hartfalen anemie zeer vaak voorkomt en gepaard gaat met mortaliteit, morbiditeit en disfunctioneren lijkt correctie met EPO een zinvolle therapie. Tegelijkertijd werd in andere studies bij het gebruik van EPO een verhoogde incidentie van beroertes gezien. Derhalve werd de RED-HF studie verricht, een grote gerandomiseerde studie waarbij patiënten met hartfalen gerandomiseerd werden naar behandeling met EPO danwel een placebo. De primaire uitkomst was sterfte door welke oorzaak dan ook of ziekenhuisopname in verband met hartfalen. In de behandelgroepen werd geen (significant) verschil in de primaire uitkomst gevonden. Echter de uitkomst "kwaliteit van leven" was bij patiënten die gerandomiseerd werden voor behandeling met EPO hoger dan in de placebo groep. Zoals eerder geopperd werd zou behandeling met EPO voor een verhoogde stollingsneiging zorgen. In de RED-HF studie wordt er geen verschil in het optreden van cerebrovasculaire accidenten bij EPO danwel placebo aangetoond, echter wel meer thrombo-embolische complicaties. In **hoofdstuk 6** geven we commentaar op mogelijk negatieve effecten bij behandeling met EPO bij patiënten met hartfalen en het therapeutische window van EPO zoals in de RED-HF studie wordt verondersteld. In **hoofdstuk 8** reageren we tenslotte inhoudelijk op de RED-HF studie.

Discussie

Anemie en cardiovasculaire ziekte

In het eerste deel beschrijven we mogelijke oorzaken van anemie bij cardiovasculaire patiënten. En belangrijke bevinding is de associatie tussen onstekingsfactoren en anemie. In de jaren negentig werd tumor necrose factor-alfa (TNF-α) ontdekt als een belangrijke biomarker bij patiënten met hartfalen. Omdat TNF-α betrokken lijkt bij ongunstige cardiale remodellering, werden klinische studies verricht, waarbij een TNF-α-remmer (infliximab) toegediend werd bij patiënten met hartfalen. Ondanks het therapeutische potentieel zorgde deze TNF-α-remmer voor een negatief effect bij patiënten met matig tot ernstig hartfalen, terwijl het effectief waarden van TNF-α en interleukine 6 verlaagde. Recente studies lieten daarnaast zien dat patiënten met een myocardinfarct geen klinisch voordeel hadden bij deze TNF-α -remmer. Helaas zijn in deze studies hemoglobine en anemie niet beoordeeld. Andere studies die infliximab onderzochten bij patiënten met andere chronische ziekte zoals reumatoïde arthritis en inflammatoire darmziekten lieten echter wel een verbetering van hemoglobinewaarden zien, wat suggereert dat TNF-α –remming een effectieve behandeling kan zijn bij patiënten met chronische ziekten. De paradoxale resultaten van eerdere studies met infliximab bij patiënten met hartfalen kunnen worden verklaard door een recente ontdekking. Uit experimentele gegevens blijkt namelijk dat twee isovormen van de TNF receptor over verschillende effecten bezitten. Myocardschade wordt bewerkstelligd door de tumor necrose factor receptor type 1, terwijl gunstige effecten gemedieerd zijn via de tumor necrosis factor receptor type 2. Infliximab, dat niet selectief een van deze receptoren inhibeert, heeft dus ambivalente effecten. Toekomstige studies moeten gericht zijn op verkenning van het mechanisme van de tumor necrosis factor receptor type en op de effecten van selectieve remming op anemie en hart-en vaatziekten.

Verder laten we zien dat verhoogde centraal veneuze druk geassocieerd is met anemie bij cardiovasculaire patiënten. Dit kan een teken zijn van vochtretentie, een typerend symptoom dat voorkomt bij hartfalen. Ten eerste zorgt een toegenomen veneuze druk dat vocht uit de capillairen de weefsels in wordt gedreven, sneller dan dat de lymfebanen dit vocht kunnen afdrijven Dit resulteert uiteindelijk in oedeem. Ten tweede ontstaat er bij chronisch hartfalen een verminderde nierdoorbloeding waardoor het renine-angiotensine-aldosteron system (RAAS) geactiveerd wordt. Dit resulteert in vochtretentie en een verhoging van het extracellulaire volume. Bovendien kunnen verhoogde waarden van het antidiuretisch hormoon zorgen voor vochtretentie.

Doordat het circulerend volume sneller toeneemt dan het hemoblobine, ontstaat er hemodilutie (verdunning), wat uiteindelijk resulteert in een pseudoanemie. Ondanks de relatie tussen anemie en vochtrentie zijn symptomen en tekenen afwezig deze studies. Het lijkt er dus op dat hemodilutie voorafgaat aan de klinische presentatie van vochtretentie. In dit proefschrift laten we daarnaast zien dat anemie in combinatie met een verhoogde centraal veneuze druk een tweemaal verhoogde kans geeft op sterfte, wat nogmaals onderstreept dat anemie een belangrijke marker is voor de mate van ziekte. Verder onderzoek naar vochtretentie en behandeling zijn om deze reden noodzakelijk. Momenteel wordt bijvoorbeeld onderzocht in een studie met een directe meting van het bloedvolume en bovendien geleide therapie met behulp van nieuwe radioactief gemerkte kleurstof verdunningstechnieken; de TEAM-HF (Treating to Euvolemia by Clinical Assessment en Measured Blood Volume in Heart Failure) studie. Een andere belangrijke oorzaak van anemie die we in dit proefschrift niet onderzochten, maar hier wel verband mee houdt is ijzertekort. Zelfs onafhankelijk van de aanwezigheid van anemie wordt ijzertekort gerelateerd aan verminderde kwaliteit van leven en een verslechterde prognose. IJzer is niet alleen een belangrijk integraal onderdeel van hemoglobine, het is een belangrijke component voor zuurstofopslag in myoglobine en betrokken bij cellulaire productie van energie in de spieren. Derhalve wordt ijzersuppletie voorgesteld als een belangrijke behandeling onafhankelijk van de aanwezigheid van anemie of als alternatief voor erythropoëse stimulerende middelen zoals EPO. Zo laat de FAIR-HF studie zien dat ijzersuppletie leidt tot een verbetering van de functionele capaciteit en levenskwaliteit. Gezien de neutrale resultaten van de RED-HF studie valt er te speculeren of anemie slechts een marker is bij kwetsbare patiënten met hartfalen. Het ijzermetabolisme zou bovendien een belangrijke schakel kunnen zijn tussen anemie en ontstekingen aangezien inflammatoire cytokines betrokken zijn bij de synthese van hepcidine. Hepcidine is een hormoon dat de ijzeropname in de darm reguleert en zorgt voor weefseldistributie door het induceren van de afbraak van de cellulaire ijzertransporter ferroportine. Toekomstig onderzoek zou zich daarom kunnen richten op deze mediatoren. Verschillende studies onderzoeken momenteel de uitkomst bij patiënten met hartfalen. In dit proefschrift tonen we aan dat, gezien de verdubbeling van de sterftecijfers, aanhoudende postoperatieve anemie niet kan worden beschouwd als een goedaardige ziekte. Om deze reden zou standaard diagnostische evaluatie en behandeling van anemie vóór ontslag de uitkomst na CABG verbeteren. Om postoperatieve anemie te beperken zou verbeterde besteding van de hedendaagse strategieën, om allogene bloedtransfusies te voorkomen, zoals minimaal invasieve chirurgie, autologe bloedtransfusies,

thrombostatische medicatie en erythropoëse stimulerende eiwitten een oplossing kunnen bieden. Ook zou aanhoudende postoperatieve anemie een marker voor een hoog risico populatie vertegenwoordigen. Daarnaast zouden patiënten die een CABG met actieve chronische ontsteking een nog hogere risicogroep vormen. Vooral deze patiënten dienen intensief te worden gecontroleerd om de duur van postoperatieve anemie na CABG te beperken. Bovendien zou het verstandig zijn om het gebruik van erythropoëse remmende factoren zoals ACE remmers in de vroege postoperatieve fase te voorkomen. Tot slot lijkt anemie een marker van de ziekte in plaats van een mediator en identificeert het derhalve de kwetsbare cardiovasculaire patiënt. Om deze reden zou anemie ten eerste als zodanig moeten worden erkend en dient er een juiste evaluatie uitgevoerd te worden, waaronder voedingstekortkomingen en reticulocyten, om de morbiditeit te verbeteren. Ten tweede, zijn nieuwe studies van belang om mechanistische oorzaken van anemie bij cardiovasculaire patiënten te onderzoeken en de morbiditeit en mortaliteit te verminderen.

Erythropoëtine en hart- en vaatziekten

In het tweede deel beschrijven we de behandeling van cardiovasculaire patiënten met EPO. Recombinant EPO wordt al meer dan drie decennia gebruikt bij patiënten met chronische nierziekte. Dit resulteert in een betere kwaliteit van leven en vermindering van bloedtransfusies. EPO werd in 2002 steeds meer erkend als een veelzijdig cytokine. In experimentele setting verbeterde EPO de infarctgrootte en linker ventrikel functie na een myocardinfarct. De eerste klinische studies van EPO therapie bij hartfalen waren veelbelovend en lieten zien dat behandeling veilig was. EPO behandeling toonde een trend naar een daling van sterfte en eerste ziekenhuisopnames in verband met hartfalen. Deze waarnemingen waren tegenstrijdig ten opzichte van andere studies met patiënten groepen waarin EPO therapie werd onderzocht. Bij patiënten met een beroerte, werd EPO bijvoorbeeld geassocieerd met een hoger sterftecijfer. Verder is in de TREAT (Trial to Reduce Cardiovascular Events with Aranesp Therapy) studie aangetoond, dat EPO bij patiënten met chronische nierziekte het risico op een beroerte vergroot. In een sub-analyse van het CHOIR (Correction of Hemoglobin and Outcomes in Renal Infufficiency) studie, ook uitgevoerd bij patiënten met chronische nierziekte, werd gezien dat dosisverhoging van EPO naar hogere waarden van hemoglobine een verhoogd risico op sterfte, myocardinfarct, congestief hartfalen of een beroerte gaf. In de RED-HF studie resulteerde chronische behandeling met EPO in frequentere thrombo-embolische complicaties, alhoewel beroerte niet vaker voorkwam in de met EPO behandelde patiëntengroep. Echter bij een myocardinfarct geeft een enkele bolus van EPO niet meer ernstige bijwerkingen. Bovendien laten we in dit proefschrift zien

dat tot een jaar na toediening EPO therapie veilig is. Vanuit deze resultaten kunnen we concluderen dat timing en dosering van EPO cruciaal is met betrekking tot de effecten en bijwerkingen. Aangezien studies met een lage dosering EPO momenteel niet beschikbaar zijn, kan het zijn dat therapie met EPO nog steeds effectief is, maar in een lagere dosering toegediend moet worden. Anderzijds worden nieuwe EPO-analogen ontwikkeld die geen hematopoëtische effecten bezitten. Deze niet-erytropoiëtische derivaten behouden weefselbeschermende eigenschappen zonder ongewenste effecten van erythropoëse. Vooral bij chronisch gebruik van deze middelen is dit wenselijk. Een recent experiment toonde aan dat behandeling met een kleine eiwit sequentie in het EPO molecuul, helix B oppervlakte peptide, leidde tot bescherming van de hartspier. Echter, dit middel wordt alleen bestudeerd door een enkele onderzoeksgroep en tot op heden ontbreken klinische studies.

De resultaten van de experimentele EPO studies laten zich niet vertalen naar de humane situatie. Verschillende factoren kunnen betrokken zijn bij deze disconcordante resultaten. Ten eerste is bijvoorbeeld de coronaire anatomie van knaagdieren anders dan bij mensen, wat mogelijk kan zorgen voor grotere infarctgrootte bij experimentele studies. Ten tweede houden experimentele studies geen rekening met comorbiditeit en medicatiegebruik. Daarnaast is dosering van EPO verschillend in experimentele setting. Ten slotte, waar EPO in experimentele setting direct na het infarct toegediend kan worden, kan dit in humane setting slechts na een mediane duur van 4 uur na aanvang van symptomen van het myocardinfarct. Het is bij de vertaling van experimentele uitkomsten naar de kliniek belangrijk zich hier van bewust te zijn.

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Curriculum Vitae

Lennaert Kleijn werd op 9 oktober 1985 geboren in Nagele. In hetzelfde dorp ging hij naar de Openbare basisschool De Ringloop en vervolgde zijn onderwijs op het Zuyderzeecollege te Emmeloord. Hier volgde hij eerst het Gymnasium om vervolgens over te stappen naar het VWO. In 2003 haalde hij zijn diploma hiervoor in het uitstroomprofiel Natuur en Gezondheid.

In 2003 begon hij aan zijn studie Geneeskunde aan de Rijksuniversiteit Groningen. Tijdens zijn studie was hij actief bij studentenwielervereniging Tandje Hoger en lid van algemene studentenvereniging Fleks. Tevens werkte hij als laboratoriummedewerker in het Universitair Medisch Centrum Groningen (UMCG) en was zodoende ook betrokken bij klinische studies en wetenschappelijk onderzoek. Zijn wetenschappelijke interesse werd verder aangewakkerd toen hij 2005 op de afdeling cardiologie begon alwaar een studentship ontving via de Junior Scientific Masterclass voor zijn proefproject “anemie bij chronisch hartfalen patiënten als gevolg van factoren in de circulatie”. Uiteindelijk resulteerde het vervolg van dit project in zijn afstudeerscriptie; “Erythropoietin in cardiovascular disease in 2007. Na het doorlopen van co-schappen in de Isala Klinieken te Zwolle, het Diaconessenhuis te Meppel en het Bethesda ziekenhuis Hoogeveen begon hij met zijn keuze-coschap cardiologie in het UMCG. In 2009 behaalde hij nominaal zijn artsenbul. Wetenschappelijk onderzoek werd voortgezet op de afdeling cardiologie in het UMCG. Na een korte uitstap om klinische werkervaring op te doen op de spoedeisende hulp in het Bethesda ziekenhuis te Hoogeveen begon hij in februari 2012 als arts-assistent cardiologie in het UMCG. Per juli 2012 is hij in opleiding tot cardioloog. Momenteel is Lennaert bezig met zijn vooropleiding cardiologie op de afdeling interne geneeskunde in het Medisch Centrum Leeuwarden. Tijdens zijn opleiding tot cardioloog werkte hij onder leiding van prof. Dr. D.J. van Veldhuisen, dr. P. van der Meer en dr. B.D. Westenbrink aan zijn proefschrift. Op 3 februari zal hij zijn proefschrift Anemia and Erythropoietin in Cardiovascular Disease verdedigen.